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PHYSIOLOGY

VASOMOTOR REGULATION

COMMUNICATION IV. SKELETAL MUSCLE VASCULAR REFLEXES INDUCED BY CHEMICAL AND THERMAL STIMULATION OF LIMB RECEPTORS

V. L. Tsaturov

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(Received November 20, 1958. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

Previous articles [1, 2] have described vascular hindlimb reflexes in which vessels to the muscles are principally involved, and which are induced by electrical stimulation of the afferent fibers of somatic nerves.

A weak electrical stimulus causes dilatation of the hindlimb vessels and a general lowering of the arterial pressure. A stronger stimulus causes contraction of the limb vessels and raises the arterial pressure. Further increase in strength of the stimulus causes a biphasic local reflex in which the initial vascular constriction is followed by a dilatation. The latter develops through an inhibition of vasoconstrictor tone [2].

Since this reaction indicates the possibility of inhibition occuring in one part only of the vasomotor center, while the other parts remain excited, it is of fundamental importance to explain the mechanism of this kind of inhibition.

The change of the local reflex from constriction to dilatation may be explained as due to the involvement of groups of afferent fibers having different thresholds and being connected with receptor having different functions.

The present article attempts to confirm this hypothesis, and describes experiments with chemical and thermal stimulation of the limb receptors.

METHOD

The cats were anesthetized with 1 g/ kg urethane and 0.03 g/ kg of chloralose, and the reflex contractions of the vessels of the lefthindlimb were recorded in terms of variations in electrical resistance. The vessels were perfused with the animal's own blood, using a special kind of pump [3] which maintained the normal minute volume of the blood at its natural level. The pressure in the femoral artery was determined by the blood-flow rate and by the resistance offered by the limb vessels. Constriction or dilatation of the vessels caused an increase respectively of the perfusion pressure; the perfusion pressure in turn indicated the extend of the constriction or dilatation of the vessels. Clotting was prevented by intravenous injection of 7.5-10 mg/ kg of heparin. The arterial pressure was recorded from the carotid artery, and a trace of the respiration was obtained from a tracheal cannula and a Marey's capsule.

The vascular supply of the hindlimb was then separated from that of the body, and the leg perfused with Ringer-Locke solution. To reduce the edema, dextran was added to the perfusion fluid. The fluid consisted of a 0.5-5 % solution of NaCl and KCl , and in some cases the NaCl concentration was increased to 10%; the receptors of the limb were stimulated by adding 10^{-6} to 10^{-2} acetylcholine or nicotine, or by using a Ringer-Locke solution satured with carbon dioxide, or by raising the temperature of the Ringer-Locke solution to 40-50°.

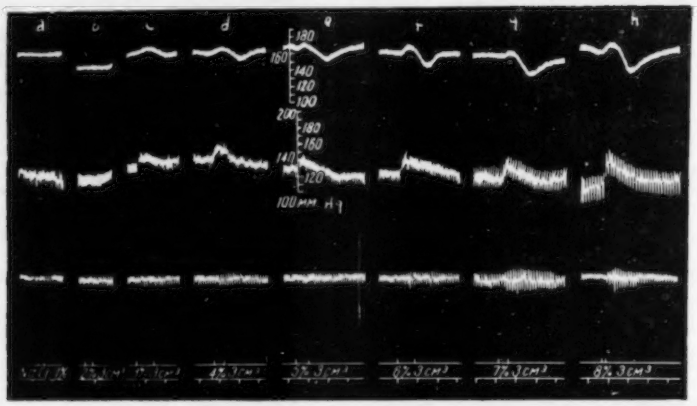

Fig. 1. Development of the reflex response to chemical stimulation of the right hindlimb by increasing concentrations of sodium chloride. a) 1%, b) 2%, c) 3%, d) 4%, e) 5%, f) 6%, g) 7%, h) 8%. Curves, from above downwards: perfusion pressure of the vessels of the left limb (resistance measurements); arterial pressure; respiration; stimulus marker; time marker (30 seconds).

The temperature of the tissues was controlled by a needle thermocouple thermometer inserted into the gastrocnemius muscle.

In this way, the receptors of the right hindlimb, which retained only a nervous connection with the rest of the animal, could be stimulated, and the reflexes affecting arterial pressure (systemic reflex) and the resistance of the vessels of the left hindlimb (segmental reflex) could be studied.

Altogether 41 experiments were carried out.

RESULTS

As can be seen from Figs. 1, a, b, stimulation with a 1 or a 2% solution of sodium chloride produces no reflex response. A 3% solution (Fig. 1, c) causes an increase in arterial pressure and a contraction of the vessels of the opposite limb. Simultaneously, there is also a small increase in the amplitude of the respiratory movements. These results are typical for all the chemical stimuli used: a threshold dose of any substance leads, as a rule, to a slight rise in arterial pressure and to a constriction of the vessels of the opposite leg. The same result is found with thermal stimulation.

In some experiments, the threshold concentrations were quite low, while in others they were much higher. However, none of the low concentrations of chemical stimuli nor any increase in temperature of the perfusate induced any reduction of arterial pressure or dilatation of the vessels of the opposite limb, such as occurs in response to electrical stimulation of a somatic nerve.

On further raising the concentration of the chemical stimulus, the segmental reflex became biphasic: the initial vasoconstriction was replaced by a dilatation (Fig. 1, d, e). Still higher concentrations caused a greater vasodilatation in the segmental reflex (Fig. 1, f, g, h). At the same time there is an increase of the pressor and respiratory reflexes.

Sufficiently strong thermal stimulation (Fig. 2, a) also causes a biphasic segmental vasomotor reflex. In this case the temperature of the muscle rose to 42°.

We found no differences in the development of the segmental reflex to result from the use of different chemical stimuli. The effects of increasing the chemical or thermal stimulus were precisely the same as those found for increasing the strength of an electrical stimulus applied to the afferent fibers.

In order to establish this conclusion more definitely, at the end of each experiment the right sciatic nerve was sectioned, and the central end stimulated electrically at 50 cps. Comparison of the segmental and systemic

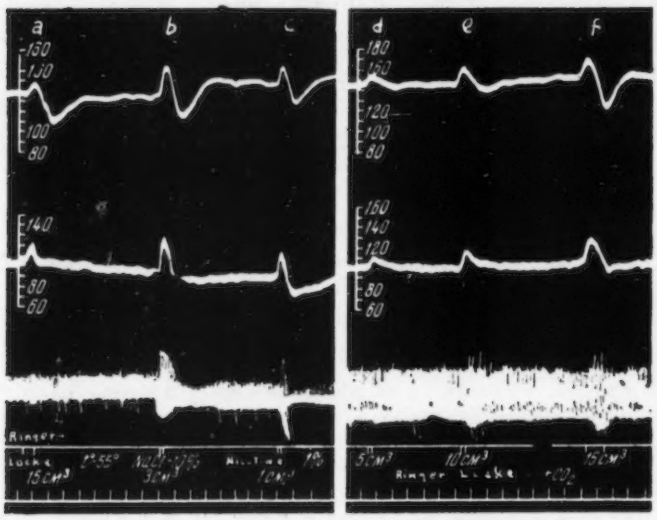

Fig. 2. Reflex response to thermal and chemical stimulation. a) 15 cm^3 of Ringer-Locke solution heated to 55°; b) NaCl— 10 %; c) nicotine 1%; d, e, f) 5, 10 and 15 cm^3 Ringer-Locke solution saturated with carbon dioxide. Curves as in Fig. 1.

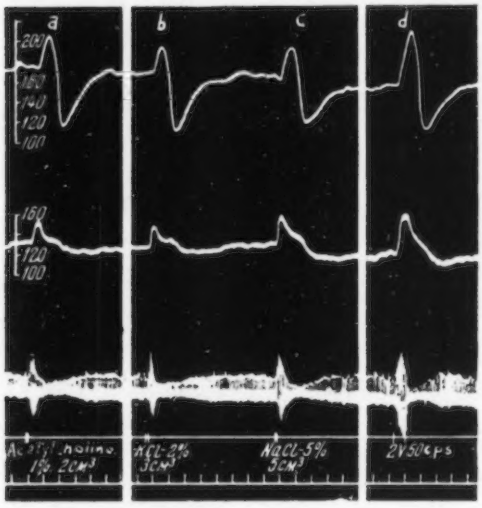

Fig. 3. Reflex responses to chemical and electrical stimulation. a) Acetylcholine —1%, 2 cm^3 ; b) potassium chloride — 2%— 3 cm^3; c) sodium chloride — 5%; d) stimulation of the central end of the right sciatic nerve. Curves as in Fig. 1.

reflexes induced by strong chemical stimulation (Fig. 3, a, b, c) with those produced by electrical sitimulation in the same animal (Fig. 3, d) shows that, broadly, the same result is obtained in the two cases.

The segmental reflexes produced by acetylcholine or by sodium and potassium chloride are almost identical with those resulting from electrical stimulation.

The same relation is also found for the respiratory reflexes. In all cases, the initial reflex hyperventilation changes over to an inhibition of respiration, and the transition occurs together with the development of the dilator phase of the segmental reflex.

A similar biphasic respiratory reaction is found in many experiments with chemical stimulation (Fig. 2, b, c). The small depressor phase at the end of the pressor systemic reflex (Fig. 2, c, Fig. 3, c) was very seldom found in our experiments.

Thus, this effect of chemical or thermal stimulation differs only in one respect from that obtained by stimulating afferent nerve fibers electrically; in the former, there is no depressor systemic reflex nor any initial vasodilatation in the segmental reflex. These responses, which are typically obtained by weak electrical stimulation, are due chiefly to the excitation of β-fibers of Group A [5, 10]. It is known that the terminal apparatus of the β-fibers has a mechanoreceptor function [8, 11].

Clearly, it is the muscle proprioceptors and the cutaneous mechanoreceptors which respond to comparatively weak mechanical stimuli. At the same time, it is known that several chemical substances, for instance acetylcholine, succinylcholine, nicotine, etc., are able to induce impulses in the fibers of these receptors [4, 6, 9].

The failure of low concentrations of chemical stimuli to elicit a depressor reflex does not mean that the receptors are not stimulated. It may be that with the chemical stimulation there is not sufficient synchronization of the afferent impulses, as in the case with electrical stimulation.

The relationship between the pressor reflex and the excitations of fibers of Groups Aδ, B and C has been demonstrated many times. [5, 10]. Since the stimulation of their receptors is affected both mechanically and by thermal and chemical stimuli [7, 11] it must be supposed that in our experiments the pressor reflex and the segmental vasoconstriction reflex resulted from stimulation of Aδ, B and C fibers.

For all the stimuli, the dilatation of the limb vessels always occurs after an initial contraction. The segmental reflex is always biphasic. For this reason, the stimuli used did not make it possible to demonstrate the existence of a specific receptor group causing only inhibition of vasoconstrictor tone. We can scarcely attribute this circumstance to lack of a sufficiently specific action of the stimuli on the receptors, because a very wide range of different substances was used.

The constant response of the vasomotor center to different kinds of stimuli indicates rather that both phases of the segmental reflex are produced by stimulation of the same receptors.

Nevertheless, the dilatation phase may be induced merely by increasing the strength of the chemical stimulus (Fig. 2, d, e, f). Although in this experiment the concentration was not changed, the time for which the receptors were stimulated was increased. We cannot therefore decide whether the biphasic segmental reflex, and particularly its dilator phase, is caused by an increase in the total number of impulses to the vasomotor center, or whether the determining factor is the duration of the afferent volley. Certain experiments with electrical stimulation favor the second alternative.

SUMMARY

One limb was separated humorally from the rest of the body, and stimulated chemically and thermally while a study of the vascular reflexes of the opposite limb was made.

The segmental vasomotor reflexes were recorded in terms of change of electrical resistance. Stimulation by sodium and potassium chloride, acetylcholine, nicotine, carbon dioxide, and by heat caused a segmental reflex in the opposite limb. With stronger stimuli, the reflexes became biphasic, so that the vasoconstriction changed to a vasodilatation.

The differences between vasomotor reflexes induced electrically and chemically are discussed.

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REFLEX EFFECTS ON ARTERIAL PRESSURE, RESPIRATION, AND LYMPH FLOW FROM STIMULATION OF THE PANCREATIC DUCT

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The pancreas is known to have both secretory and excretory functions. The abundant innervation of this gland has been repeatedly demonstrated [2, 3, 4, 6, 7]. Many authors [5] have supposed that the acinous and islet tissues are independently innervated. It has been shown physiologically [8] that stimulation of the intact pancreatic nerve and also the central and peripheral ends of the nerve after it has been divided, causes both pressor and depressor responses.

An increase or decrease of the pressure in the blood vessels of the pancreas has been shown [1] to cause general reflex changes in arterial pressure, and sometimes an increased respiration.

We have found no published accounts of work on interoceptor reflexes from the pancreatic ducts.

In the present work, we have attempted to study the effects of interoceptor stimulation of the pancreas on arterial pressure, respiration, and lymph flow.

METHOD

The experiments were carried out on adult dogs under morphine-urethane or morphine-pentothal anesthesia. The abdominal cavity was opened, and the lymphatic vessels and the small arteries from it were ligatured. The only remaining connection of the gland with the rest of the body was through the nerve and the large blood vessels. A cannula was inserted into one of the pancreatic ducts, and the other was tied off.

A study was made of reflexes induced by increasing the pressure in the duct with injections of Tyrode's solution heated to 37-38° and given through a Janet syringe. In some of the experiments, the pressure in the duct was recorded by a mercury manometer. The pressure was raised to 120-160 mm of mercury. In many of the experiments, the receptors were stimulated while the gland was perfused.

Recordings were made of the arterial pressure in the carotid artery, the respiration being registered by a Marey's capsule connected to a side tube of a cannula inserted into the trachea; traces of the stimulus were also made, and a time marker was used to indicate 5-second intervals. Lymph flow was recorded by inserting a cannula into the thoracic lymph duct, and counting the drops with a drop counter. An intravenous injection of 208 international units per kg body weight of heparin was given to prevent clotting.

Nineteen experiments were carried out, including 45 sets of observations, and 5 experiments were carried out during artificial respiration.

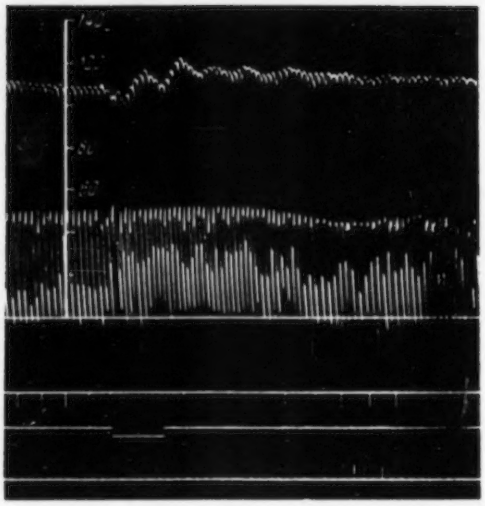

Fig. 1. Changes in the arterial pressure, respiration, and lymph flow on increasing the pressure in the pancreatic ducts. Curves, from above downwards: arterial pressure, respiration base line, lymph flow, stimulus marker, time marker (5 seconds).

RESULTS

Out of the 45 observations, after increasing the pressure in the duct to 120-160 mm of mercury, in 27 cases there was an increase in arterial pressure, in 4 a decrease, and in 2 cases the initial fall was rapidly followed by a rise; in 1 case there was an initial rise followed by a fall, and in 3 instances there was a reduction of the pulse amplitude, while in 8 it remained unchanged. The changes in the respiration rate were very varied. In 21 cases the amplitude increased, and in 3 of these, besides an increase in the thoracic movement there was also an increase in the rate, while in 2 others it slowed down; on 6 occasions the amplitude was decreased, and in 4 cases the rate was also reduced; on 2 occasions there was an increased rate of respiration, in 2 a decrease, and in 7 others there was no change.

Together with the changes in arterial pressure and respiration just described, a reduction in lymph flow was observed 35 times, an increase 3 times, while on 7 occasions there was no change.

Traces of some of the experiments are given in Figs. 1 and 2.

In Fig. 1 it can be seen that increasing the pressure in the ducts causes a rise in arterial pressure from 100-110 to 114-122 mm of mercury, as well as an increase in respiration rate and a marked rise in lymph flow. We assume that these three changes are reflex.

It has already been shown that in certain cases an increase in arterial pressure and thoracic respiratory movements can cause a change in lymph flow. However, in the case described, and in most of our experiments, in spite of the observed changes in arterial pressure and respiration which might be expected to cause an increase in lymph flow, actually a decrease was obtained.

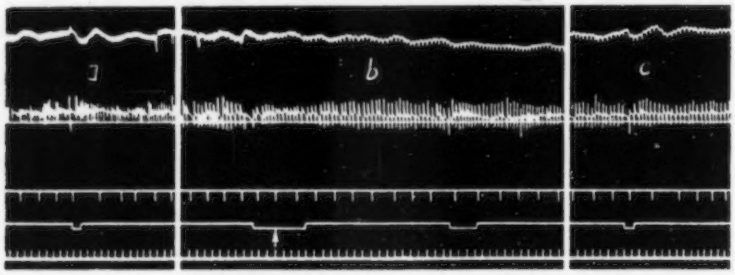

Fig. 2. Variation of arterial pressure, respiration, and lymph flow on increasing the pressure in the pancreatic duct to 140 mm mercury. a) Before injecting novocain into the duct; b) 2 minutes 5 seconds after the injection; c) 21 minutes after the injection. ↑) Moment of novocain injection (4 ml 0.25%). Curves as in Fig. 1.

In many cases, it would appear at first sight that the change in lymph flow resulted from a reduction in the rate or in the depth of respiration. However, this explanation is probably not correct, because in our experiments, a reduction in lymph flow occurred during the operation of such important factors as increase in the arterial pressure, and sometimes by a motor reaction occuring together with an increased depth and frequency of respiration, factors which under certain conditions lead to an increased lymph flow.

Also, a reduced lymph flow was found in 18 cases to be associated with an increased depth of respiration, in 6 to occur without any respiratory change, in 2 to be associated with an increased respiration rate, while in 5 cases the respiration was maintained artificially.

To confirm that the changes in arterial pressure, respiration, and lymph flow in response to the stimuli used were reflex in nature, a number of experiments were arranged in which 3 ml of 0.25% novocain was injected into the duct. After the injection, no change in arterial pressure, respiration, or lymph flow occurred, as can be seen from Fig. 2.

After increasing the pressure in the duct to 140 mm, a small increase in arterial pressure was observed, after which it fell. The respiration became deeper, and the lymph flow became markedly reduced (Fig. 2, a). Increasing the pressure in the ducts immediately after injecting them with 4 ml 0.25% novocain solution caused no alteration in arterial pressure, respiration, or lymph flow (Fig. 2, b). After the effect of the novocain had worn off, the arterial pressure, respiration, and lymph flow once more responded to alteration of pressure in the duct (Fig. 2, c). It seems to us that the changes occur as follows.

From the classical work of I. P. Pavlov and his school, it is known that there is an increased secretion of pancreatic juice in response to the entry of chyme from the stomach into the duodenum. It may therefore be supposed that the gastric juice, which exerts a certain pressure on the sensitive elements of the duct, causes their stimulation. Impulses from the baroceptors exert what is evidently a reflex inhibitory effect on intestinal peristalsis. It is also known that intestinal movements have a considerable effect on lymph flow. Since approximately three quarters of the total amount of lymph entering the venous system from the thoracic duct is intestinal lymph, it may be supposed that the reduction in lymph flow observed in our experiments was not only the result of reflex change in the size of the lumen of the large lymphatic vessels, but also resulted from some inhibition of intestinal peristalsis. This idea certainly requires to be tested experimentally.

SUMMARY

The experimental result indicated that baroceptors were probably present in the pancreatic ducts, whose reflex stimulation caused changes in arterial pressure, respiration, and lymph flow. These reflexes were eliminated by injection of novocain into the ducts.

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* In Russian

THE EFFECT OF STATIC EFFORT ON BLOOD DISTRIBUTION IN A HEALTHY HUMAN SUBJECT

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(Received December 15, 1958. Presented by Active Member of the AMN SSSR V. V. Parin)

A great many reports have been made of plethysmographic determinations of blood distribution during muscular work.

Weber [7, 8] has proposed that the functional condition of the cardiovascular system can be determined by making plethysmographic measurements on the limbs when performing measured amounts of muscular work by flexing and extending the foot. He reports that in a healthy subject there is a rise in the curve ("positive" plethysmogram) during such work on account of a dilatation of the vessels. When fatigue sets in, the vascular reaction changes, so that the vessels now contract ("negative" plethysmogram). According to him, a change in this curve following a work period of 10-15 seconds indicates a defect in the cardiovascular system.

However, this method has not been brought into general use, because the "negative" has been found to occur sometimes during work by healthy subjects not leading to fatigue [6, 9]. Even Weber himself has shown that in very nervous subjects, a negative plethysmogram may occur during muscular work. All the same, most investigators consider that dynamic work leads to dilatation of the blood vessels [1, 2, 4, 7, 8].

Comparatively little attention has been paid to the distribution of the blood during static effort. Recordings of the tension developed by clenching the first with maximum force for 3 minutes [5] showed three kinds of curves: of these, 93 plethysmograms (82%) belonged to the first type. After the tension had developed, there was a constriction of the blood vessels lasting for 30-60 seconds, and subsequently the curve rose, showing the vessels had dilated.

A. G. Zima [3] showed that when the rubber bulb of a manometer was compressed by hand, at the beginning of the static effort there was a contraction of the vessels of the opposite forearm. As fatigue developed, and long before the end of the effort, the curve gradually rose, in some cases returning to its initial level. It must be noted that the rise in the curve, corresponding to a dilatation of the vessels, begins essentially after the end of the static work. When a dumbbell was held in one hand, and static work was carried out by the opposite leg bent at the knee joint; even at the onset of the work, a considerable dilatation of the vessels was observed. Unfortunately, Zima gave no value for the static effort or for the number of experiments carried out.

In static work by the muscles of the forearm, the dilatation of the vessels of the opposite arm does not occur during the working period, but after it. However, after many repetitions, the onset of the dilatation shifts until it occurs at the onset of the work, just as in dynamic work [1].

We have studied alterations of the plethysmogram occurring during different degrees of static effort involving both small and large groups of skeletal muscles.

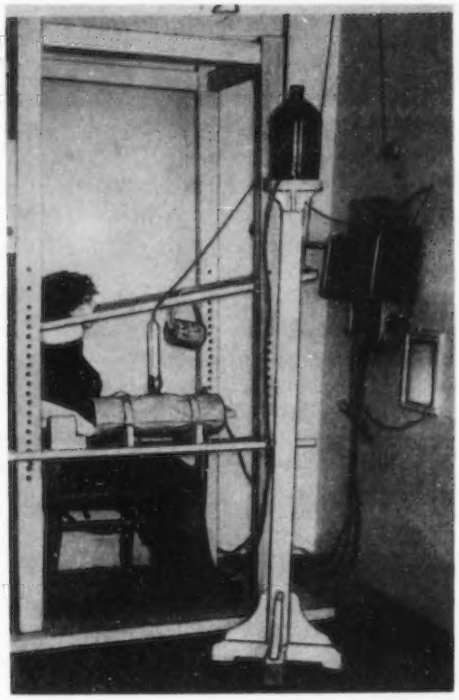

Fig. 1. General view of the stand for studying the vascular reaction during static effort.

METHOD

The investigations were carried out using a Mosso-Novitskii plethysmograph applied to the arm. The plethysmogram of the right arm was recorded to show the response to static effort of different groups of muscles. In some experiments, the pneumogram was recorded simultaneously. The subjects were placed in either the sitting or the standing position, and carried a load equal to one third, one half, or the whole of the body weight; with their left hand they compressed the rubber bulb of a mercury manometer with a force equal to 1/6, 1/4, 1/3, or 1/2 of the maximal effort and maintained the pressure until their strength gave out. Also, another set of observations was made in which a maximum effort was made with the flexors of the foot.

In order to record the plethysmogram with the subject in different positions, and for the experimenter to be able to increase or decrease the load from his own separate room, the subject was placed in a specially constructed stand (Fig. 1). This arrangement enabled external stimuli to be eliminated.

In the first tests, the subjects learned to maintain a fixed body position, and recordings were made of the so-called "zero" plethysmogram, which served as base line.

The verbal announcement by the experimenter that the work was to begin was by itself sufficient to cause a reaction in which the blood vessels contracted. To eliminate this verbal stimulus, the subject pressed the rubber bulb of the mercury manometer without any signal being given 2-3 minutes after the onset of recording the plethysmogram, and raised the mercury column to the required height. When this was done, the electrical circuit was closed, thereby recording the onset of the static work. As soon as the subject ended the work, and the mercury column fell, the contact broke and the end of the working period was recorded.

In the first observations with a load, it was found that the actual procedure of placing the weight on the shoulders was itself a powerful stimulus. Many of the subjects became agitated at this moment. We therefore caused an extinction of this vascular reaction to placing a load on the shoulders.

Twenty-five medical students aged 19-20 years were used as subjects, and 205 plethysmograms showing the response to static effort were made.

RESULTS

Static contraction of a large group of muscles (carrying various load on the shoulders) causes a dilatation of the arm vessels.

With a moderate load, in which the subjects held a load equal to half the body weight on the shoulders, out of 59 subjects, in 53 a dilatation of the vessels occurred at the onset of the effort, and was maintained during the whole of the muscular contraction period. Usually, the greatest dilatation was observed in the first 3-4 minutes after the load had been applied. When the static effort ceased, the vessels gradually returned to their normal size (Fig. 2). In 5 subjects, there was no vascular reaction to the load, and in 1, there was a biphasic plethysmogram in which at the onset of the effort the vessels contracted and then later dilated.

When repeated tests were made with the load, in some subjects, merely being placed in the special stand caused approximately the same reaction as did carrying the load. The dilatation of the vessels before the static

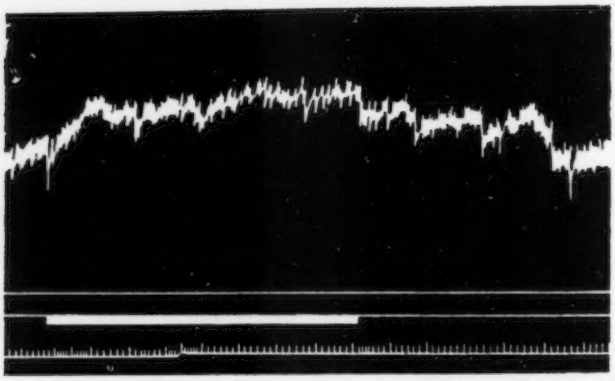

Fig. 2. Changes in the plethysmogram during the carrying of a load equal to half the body weight on the shoulders. Curves, from above downwards: plethysmogram; base line; application of static load; time marker (10 seconds).

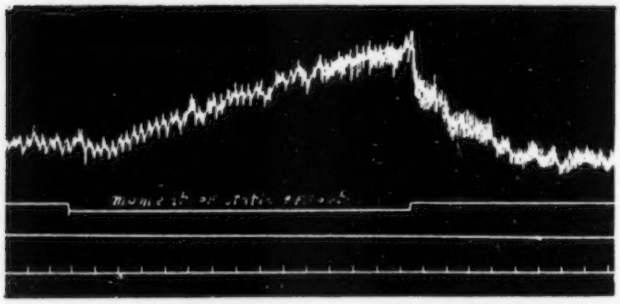

Fig. 3. Changes in the plethysmogram following a squeezing movement with the hand recorded by a mercury dynamometer; ⅓ maximal effort. Curves as in Fig. 2.

effort was considerable, and after the application of the load, the additional dilatation was not great. We are inclined to interpret this reaction as a conditioned reflex developed by association with the experimental setup. This view is confirmed by the fact that besides this reaction which occurred before the work began, there was also an increase in the heart rate of 6-12 beats per minute. This phenomenon is clearly similar to that found in athletes before the beginning of an event.

With large loads up to two thirds of the body weight, the change in the size of the vessels is the same as for a medium load, i.e., the vessels dilate, but the change is more marked.

When the bulb is squeezed with one third of the maximal effort, in 22 out of the 30 trials, there was a dilatation of the vessels of the arm. The reaction occurred either immediately after the static effort, or 15-40 seconds afterwards (Fig. 3). The greatest vascular dilatation occurred during the second half of the muscular effort, and the plethysmogram reached its peak before the end of the work. Releasing the muscular tension caused a marked drop in the curve, and within the first minute it returned to the base line. In 8 tests there was either a constriction of the blood vessels or else a biphasic plethysmogram trace was obtained.

When half the maximal effort was applied to the dynamometer, in 33 out of 41 cases, the typical vascular dilatation was observed. When the reaction did not occur immediately, the delay was less than in cases when one third of the maximun effort was exerted, the time interval being usually 5-25 seconds. The extent of the dilatation was greater and developed more rapidly. Only 2 cases of vascular constriction occurring were observed. Six biphasic curves were obtained in which the line first fell, and then rose.

When one quarter of the maximum force was exerted, at first there was no reaction of the vessels, and then a gradual dilatation occurred. With a still smaller static effort of one sixth of the maximal value, change in the blood volume of the hand was negligible, though even here there was some slight dilatation.

Just as when a load was carried on the shoulders, when muscular tension was developed by a small group of muscles, in this case the flexors of the hand, using one third or one-half the maximal tension, in some cases a conditioned reflex dilatation occurred before the effort was made.

When maximal compression of the bulb was maintained by the foot for 3-5 minutes, usually a biphasic curve was obtained in which there was first a constriction and then a dilatation of the blood vessels.

Thus, the results of these observations show that different static efforts by both large and small muscle groups usually lead to a dilatation of the arm vessels. This dilatation begins either immediately or shortly after the effort is started. When the flexors of the foot exert their maximal effort, the vascular reaction is biphasic. Such a response is evidently associated with the fact that when making a maximum effort, there is a marked excitation of the sympathetic system. With smaller static efforts, this sympathetic effect does not usually occur.

Evidently, such factors as the condition of the subject and the excitability of his cardiovascular system, as well as the conditions under which the test is carried out, have an influence on the result. Thus, for instance, we found that if the task was carried out while the subject was in an excited condition, either before or after an examination, either there was no vascular reaction, or else the vessels contracted.

The atypical vascular reaction to static effort, which may take the form simply of a contraction or else of a contraction followed by a dilatation, is found chiefly in excitable subjects. Tests have shown that in these subjects there was a preponderance of excitatory over inhibitory processes. Usually, the atypical reaction appeared only during the first few performances with a static load, and later the usual dilatation reaction developed. It must be pointed out that in excitable subjects the vascular constriction occurred not only when working with static loads, but also during work involving muscular movements, such as rhythmically squeezing the dynamometer.

When using the plethysmograph, it is important to realize that every new stimulus induces the usual vascular constriction which is a component of the orienting reaction. Therefore, before studying the plethysmographic changes due to muscular work, prelimiray experiments must be made in order to extinguish any effect which may result from the experimental setup on the vessels and to establish the base line for the plethysmogram.

It might be thought that the actual work itself could constitute a new stimulus, and might induce a vascular reaction as a component of the orienting response. Therefore, before the main observations are made, the subject must become familiar with the kind of effort which he will have to carry out. Some such experience of the work to be done is absolutely necessary. This view is borne out by some observations we made on 9 subjects who had never worked with a plethysmograph before, and had never used a dynamometer statically. It was typical, that in 5 of these inexperienced persons, when exerting one-half the maximal effort, an atypical reaction was obtained in which either there was a vascular constriction or a biphasic response. During this reaction, in two cases a change in the breathing rate during the exertion of the effort evidently influenced the result. A record of the pneumogram from the upper region of the abdomen showed that in these experiments there was a disturbance of the respiratory movements and that a considerable tension was developed.

The variation in the results obtained by different workers on the effects of a static effort, may be,to a large extent,due to different methods of investigation.

Atypical vascular reactions must not be interpreted as indicating any abnormality of the vasomotor center, since they are evidently associated in healthy subjects with individual characteristics of the nervous system.

In static work, we never found there was a change from a positive to a negative plethysmogram. On the contrary, when the effort ended, when fatigue was at its maximum, there was a maximum increase in blood volume. The response of the vessels to a steadily maintained tension of the muscles depends primarily

upon reflex effects; the conditioned reflex dilatation which occurs before work is commenced serves as an example of how the human organism prepares itself for muscular work which is about to be undertaken. It must, however, be realized that humoral factors are also concerned in these vascular changes. In certain cases we found that at the start of the work, there was no reaction, and the change in the blood vessels occurred only after the muscular tension had been maintained for 10–40 seconds.

Our investigations have shown that changes in the blood volume of a limb whose muscles are maintaining a steady tension are in the same direction as those which occur when changes in muscle length occur and external work is done.

SUMMARY

Static efforts of various strength, developed by small and large groups of skeletal muscles, provoke vascular dilatation in the hand; it starts directly or some time after the commencement of the effort. With the maximal static effort the vascular reaction was found to be biphasic: the vessels being first constricted and then dilated. Atypical reactions of the vessels in static efforts are revealed mainly in the excitable subjects during the first observation. The reaction of the vessels in static efforts depends mainly on the reflex influences. In a number of cases conditioned reflex dilatation of the vessels was observed. Humoral factors apparently are also involved in the vascular reaction.

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THE NATURE OF SPINAL SHOCK

COMMUNICATION III. THE STATE OF THE AFFERENT NEURONES

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According to our results, spinal shock develops as a consequence of lack of tone in motoneurones which have lost their ability to become excited as a consequence of the rapid separation of the spinal nervous centers from the usual supraspinal tonic impulses. During this atonic period, the neurones are in a condition of anelectrotonus or hyperpolarization, and all the agents such as potassium chloride, eserine, proserine, and strong, maintained stimulation, which act parabiotically, prevent this lack of motoneurone tone from developing, and so oppose the development of spinal shock [3, 4 and 5]. However, the alternative view has been put forward [1, 2] that spinal shock represents a protective inhibition developing in the sensory part of reflex arcs as a result of their traumatic excessive stimulation. This protective inhibition passes through parabiotic phases.

The first set of experiments reported here was devoted to comparing the duration of the traumatic impulses in the pathways of the spinal cord and in the motoneurone after chordotomy. The second set of experiments was designed to determine the functional condition of the afferent section of the reflex arc during spinal shock.

METHOD

The experiments were carried out on frogs (Rana chensinensis) which were maintained in fixed position on a special stand. A two-channel cathode ray oscillograph was used to record the impulses from the posterior and lateral columns, in the central ends of the cut dorsal roots, and, in some experiments, in the muscles also. In all cases bipolar electrodes were used. The usual platinum electrodes were employed to measure the duration of the impulses in the nerve roots. In most experiments the uninsulated portion of the electrodes was 30 μ in diameter, and that of the insulated part 50μ. They were inserted into the columns of the spinal cord, dorsal roots, or muscles by a micromanipulator.

RESULTS

The first set of experiments enabled the duration of the traumatic impulses in the dorsal and lateral columns and the duration of the excitation in the ventral roots and semitendinosus muscle to be determined (Table 1).

The traumatic impulses passing along the posterior and lateral columns continued for approximately the same time, so that both these quantities can be considered together. For the same reason, the durations of the excitation recorded in the anterior roots and in the muscle (Table 2) can also be combined.

Tables 1 and 2 show that the length of time for which impulses flow in the axons of the posterior and lateral columns after chordotomy is very short, and has an average value of 0.88 seconds, varying between 0.15 and 2.7 seconds. On average, the discharge of the motoneurones lasts 6 times longer. This longer duration of 1.5-12-5 seconds is due to the closed circulation of impulses passing from the proprioceptors by the direct and indirect routes through internuncials to form segmental arcs, so causing a delay in the discharges in the cord. These features are illustrated by the oscillogram shown in Fig. 1.

TABLE 1

Duration of Traumatic Impulses in Chordotomy (in seconds)

Site from which traumatic impulses were recorded	Number of experiments	Time for which impulses occurred		
		minimum	maximum	average
Posterior columns	11	0,15	2.7	0,79
Lateral "	4	0,4	2.0	1,1
Ventral roots	15	1,55	10,0	5,33
Semitendinosus muscle	23	1,5	12,5	5,7

It was not possible to correlate the duration of the traumatic discharge in the cord or the excitation of the motoneurones with different functional conditions resulting from chordotomy (spinal shock, unchanged condition of reflexes, or exaltation of spinal reflexes) (Table 3).

The discharge in the motoneurones lasts for 5.5 seconds in spinal shock, but when chordotomy is followed by the condition of exaltation, it continues on average for 7 seconds. Therefore, these results may indicate that the more prolonged and greater degree of excitation of the motoneurones does not help, but rather hinders the development of spinal shock.

TABLE 2

Duration of Traumatic Discharge Caused by Chordotomy (in seconds)

Site from which traumatic impulses were recorded	Number of experiments	Time for which impulses occurred		
		minimum	maximum	average
A. Axons of the lateral and of the posterior columns.....	15	0,15	2,7	0,88
B. Anterior roots and muscle....	38	1,5	12,5	5,56

In the second set of experiments, we recorded afferent touch impulses induced by stimulation of the ventrolateral surface of the left foot of the frog by an automatic touch stimulator using a soft brush. The left anterior nerve roots were sectioned in order to exclude proprioceptor impulses. In 20 experiments, impulses from cutaneous receptors were picked up by electrodes inserted into the left ninth dorsal root ganglion close to its origin from the cord, while in 4 experiments these impulses were led off from the dorsal column. On the right side, where all the roots remained intact, a flexor reflex was was induced by stimulating the foot with a coarser brush. The contraction of the biceps femoris muscle was made electrically.

All 24 experiments showed that spinal cord section has no effect on the functional condition of the afferent link of the reflex arc. Whether the chordotomy resulted in spinal shock, as it did in 14 experiments, or whether the reflex excitability of the spinal cord was raised, as in 2 experiments, or whether the excitability was unchanged, the discharge from the cutaneous receptors remained unaltered both in the dorsal root ganglia and in the dorsal columns. The results are illustrated by the oscillograms from two experiments (Figs. 2 and 3).

The condition of the afferent pathways after chordotomy can be determined only by using a completely standardized method of stimulating the receptors. In our experiments, it was essential to exclude impulses from the proprioceptors, because the changed condition of the muscles resulting from chordotomy inevitably affected the sum total of the impulses in the dorsal roots and columns. Therefore, before the experiment was started, in frogs, the VIII-XI anterior roots on the left side were cut, and the electrode inserted into a position in the IX posterior root or column, such that touching the left foot produced a clearly shown volley of impulses.

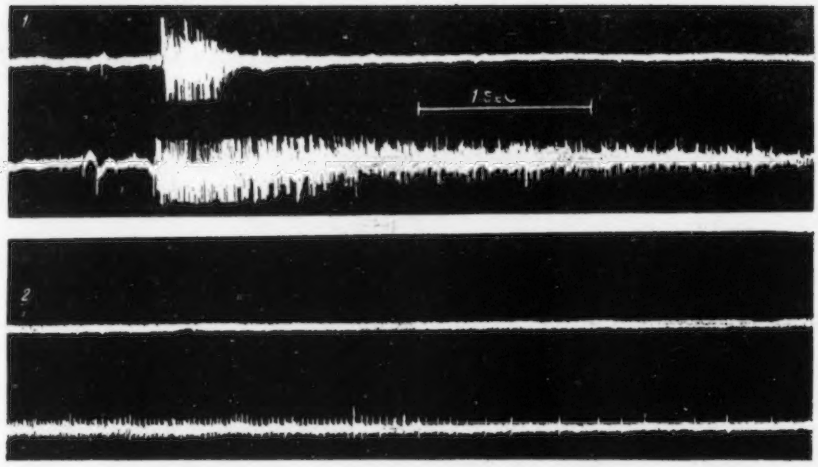

Fig. 1. Duration of discharge following chordotomy. Experiment No. 25. Curves, above downwards: nerve impulses in posterior column of spinal cord, same in central end of right ventral root. 1) Nerve impulses during chordotomy ; 2) continuation of the upper oscillogram.

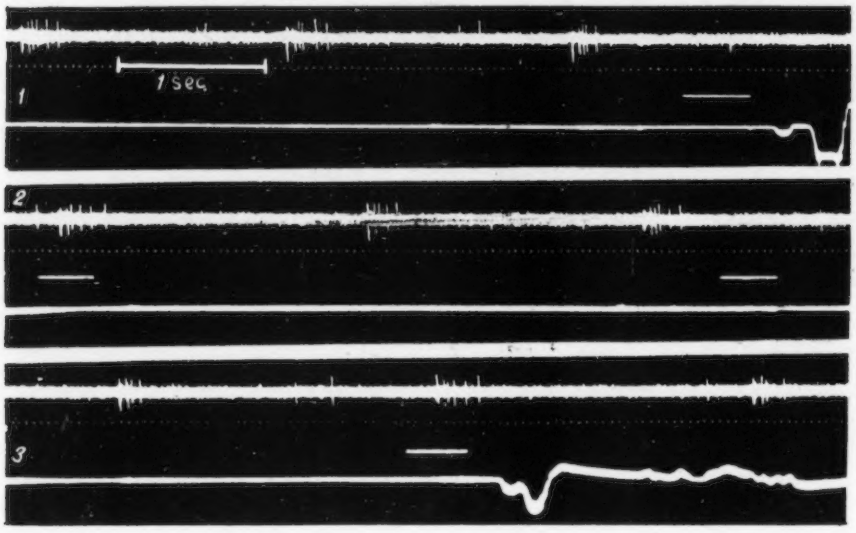

Fig. 2. Recording of afferent impulses in posterior root during chordotomy. Experiment No. 67. Curves, from above downwards: impulses in left posterior root on stimulating skin of foot automatically with a touch stimulator, trace showing stimulus to right foot, electrical record of reflex contraction of right biceps femoris. 1) Initial condition, before chordotomy ; 2) immediately after chordotomy, no reflex, spinal shock ; 3) 4 minutes after chordotomy, reflex returns.

The touch stimulator was operated by a small wheel with three teeth. The three touch stimuli were not absolutely identical, but they were repeated in a consistent order. From Fig. 2, it can be seen that the duration of the volley in the posterior root, the grouping of the impulses, and even the number in a volley, as produced by the successive stimuli, are almost exactly the same in the three oscillograms taken before chordotomy, during spinal shock, and after the restoration of the reflexes. The recording of the right biceps femoris contraction shows the state of excitation of the spinal cord existing at the time the afferent volley was recorded.

TABLE 3

Comparison of the Duration (in seconds) of the Traumatic Discharge with the Functional Condition of the Spinal Cord after Chordotomy

Functional condition after chordotomy	Duration of discharge					
	spinal tracts			motoneurones		
	minimum	maximum	average	minimum	maximum	average
Spinal shock	0,15	1,4	0,6	1,5	9,9	5,5
No change	0,4	2,7	2,2	2,1	10,0	4,9
Exaltation	0,5	0,55	0,52	6,4	7,5	7,0

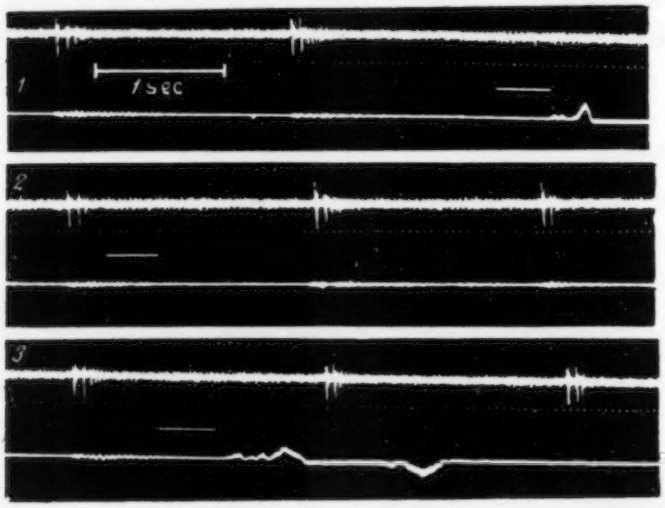

Fig. 3. Afferent impulses in posterior column of spinal cord in chordotomy. Experiment No. 71. Curves, from above downwards: nerve impulses in left dorsal column of cord on stimulating skin of foot automatically with a touch stimulator; other features as in Fig. 2. 1) Initial condition before chordotomy; 2) immediately after chordotomy, no reflexes, spinal shock; 3) 3 minutes after chordotomy, reflex returns.

Fig. 3 gives traces in which the afferent impulses were recorded from the posterior columns. In this case too, the impulses retain the same features when recorded during spinal shock, and they are not changed later when the spinal reflexes become restored.

Thus, our experiments give no support to the suggestion that spinal shock is the result of a protective inhibition developing in the sensory portion of reflex arcs due to traumatic over-stimulation.

SUMMARY

A study was made of the effect of chordotomy on voltages recorded from the posterior and lateral columns of the spinal cord, from the anterior and posterior nerve roots, and from certain muscles.

It was shown that section of the cord causes a discharge of impulses in the posterior and lateral columns which lasts on average for 0.88 second. The average period of excitation of the motoneurones is six times longer, and it is thought that this is due to the circulation of impulses in segmental reflex arcs. No connection was found

between the period for which the impulses were generated and the condition of the spinal reflexes obtained after the chordotomy. Spinal shock is not the result of prolonged stimulation. A study of the impulses generated by a standardized cutaneous stimulation showed that they remained unchanged in all parts of the afferent pathways of the cord.

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CHANGES IN FILTRATION, REABSORPTION, AND RENAL OUTFLOW IN DOGS GIVEN WATER BY VARIOUS ROUTES

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In studying water diuresis, we have become interested in changes occurring in the kidneys in response to giving large amounts of fluid. Determination of glomerular filtration and reabsorption in the tubules has shown which process accounts for the increased diuresis occurring after normal drinking, or after parenteral injection of fluid. Also, because urinary excretion is closely associated with renal blood supply, we have studied the effect of a water load on the renal circulation.

Most authors hold that in water diuresis there is a reduced reabsorption, while filtration does not increase, and may even be reduced [5, 6, 11]. Other workers have observed both a reduced reabsorption and an increased filtration [7, 8]. H. Ludemann and his co-workers [9], who worked with large water loads, ascribed the increased diuresis chiefly to an increase in the filtration rate.

The few published reports on alterations to the renal circulation resulting from water diuresis are based mainly on acute experiments. A. Walker and his co-workers [12] measured the blood flow through the kidney with a Rein stromuhr, and collected the urine by a catheter; they found that renal flow plays no part in the increased diuresis resulting from a water load or from mercury diuretics, and is only slightly increased after the injection of purines. V. V. Zakusov [4] who used the same method, found there was an increased renal blood flow and diuresis in response to diuretics, but discovered no definite relationship between them. We have read no published accounts of results obtained in chronic experiments. Very little work has been done on changes in the filtration rate, tubular reabsorption, and renal circulation induced by a parenteral injection of fluid.

METHOD

The investigations were carried out on dogs with ureteric fistulas. The urine was collected, as a rule, at 15 minute intervals, ans it was therefore possible to observe changes in the reabsorption and renal circulation. In most of the experiments, the water load consisted of 30 ml per kg body weight of water mixed with 10 % milk; intraveous injections of isotonic sodium chloride solutions were given, and in other experiments glucose solutions were used; the volume of fluid injected was 20–25 ml per kg body weight, and it was given at a rate of 20 ml per minute.

The filtration and reabsorption were measured by the creatinin method. The urine creatinin was determined by the well-known Folin method, while for the blood creatinin (endogenous creatinin) we used our own modification of the method [1]. The renal blood flow, or more accurately the "plasma flow" was measured by the use of phenol red. We found no published method of measuring renal blood flow in animals using phenol red, and therefore in collaboration with E. V. Chaikovskaya [2] we first worked out an appropriate method using a

TABLE 1

Effect of a Water Load on Filtration and Reabsorption (dog Damka, experiment No. 2, April 12, 1952)

Time (hours min)	Amount of urine (in ml)		Concentration of creatinin (in mg %)		Concentration index	Filtration (in ml/min)	Reabsorption	Filtrate, excreted in urine
	total	per min	in urine	in blood			in percentage	
10.00—11.00	12	0,20	168,1	1,02	164,70	32,94	99,39	0,61
(Water load of 500 ml given at 11 a.m.)								
11.30—	57	1,90	37,0	—	36,27	68,91	97,24	2,76
12.00	122	4,07	13,5	--	13,92	56,65	92,82	7,18
12.30	91	3,03	13,9	0,97	14,33	43,42	93,02	6,98
13.00	62	2,07	31,2	—	32,15	66,65	96,89	3,11
13.30	40	1,33	18,9	—	19,49	25,93	94,87	5,13
14.00	14	0,47	34,2	—	34,20	16,07	97,08	2,92
14.30	31	1,03	38,8	1,00	38,80	39,96	97,42	2,58
15.00	13	0,43	51,8	—	51,80	22,27	98,07	1,93
15.30	6	0,20	102,0	—	102,00	20,40	99,02	0,98

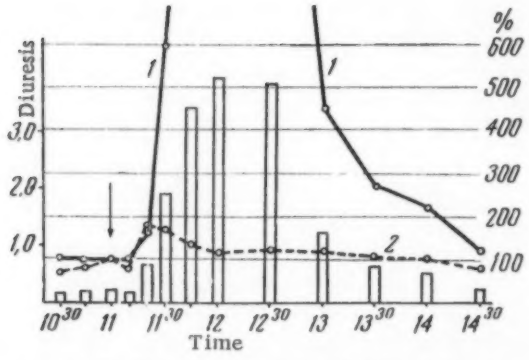

Fig. 1. Effect of a water load on filtration, reabsorption, and diuresis (dog Astra, weight 17 kg). 1) Portion of filtrate not reabsorbed, expressed as percentage of original value; 2) filtration (in ml per minute) as a percentage of initial value; arrow — dog drinks 500 ml of water; columns — diuresis in ml/minute.

single injection of a suspension of the dye in oil. Altogether, 52 experiments were carried out on 14 dogs; 227 measurements of filtration and reabsorption and 185 determinations of renal blood flow were made.

RESULTS

Experiments with a water load showed that diuresis occurred together with a marked reduction in reabsorption; in some cases the reduction was from 99-98 to 93-89%, and accompanied a 7-to 10-fold increase in diuresis. In most instances the filtration varied over the normal limits, or else increased, but this had a very much smaller influence on the diuresis. No reduction in filtration after a water load was observed.

Table 1 shows the results of one of the experiments. During the period of maximum diuresis, at 12 o'clock, the filtration had increased 1.7 times, but the reabsorption had fallen from 99.4 to 92.8% (the fluid which had been filtered and not absorbed increased from 0.6 to 7.2%, i.e., by 12 times). Thus, the increased filtration would correspond to an increase in diuresis of 1.7 times, while the reduced reabsorption would lead to a rise of 12 times, making 20 times in all; these figures agreed with the amount of urine collected.

Fig. 1 shows graphically the results of one of these experiments, in which the urine was collected at 10-minute intervals during the first half hour after giving the water. For the sake of clearness, instead of showing the amount of reabsorption as a percentage, the percentage of the unabsorbed filtrate is plotted, i.e., the amount excreted with the urine. It can be seen that the increased diuresis results chiefly from the marked reduction in reabsorption, although in the first 30-45 minutes, there is also an increased filtration.

In the first 15-30 minutes, in most experiments, renal blood flow increased considerably. Subsequently, it was was maintained at a fairly constant rate, or, more frequently, it fell in spite of the maintained high rate of diuresis which reached its maximum level at the end of the first hour (Fig. 2).

TABLE 2

Effect on Filtration and Reabsorption of Injected Physiological Saline (dog Krasotka, experiment , January 12, 1954)

Time (hours min)	Amount of urine (in ml)		Concentration of creatinin (in mg%)		Concentration index	Filtration (in ml per min)	Reabsorption	Filtrate excreted in urine
	total	per min	in urine	in blood			Percentage	
11.00 —								
11.15	8	0,53	57,14	—	55,47	29,40	98,20	1,80
11 30	9	0,60	59,26	—	57,53	34,52	98,26	1,74
11.45	7	0,47	60,61	1,03	58,84	27,65	98,30	1,70
Between 11: 35 and 11: 50 a.m., 300 ml of 0,85% NaCl solution was given intravenously								
12.00	31	2,07	37,50	—	36,41	75,37	97,25	2,75
12.15	34	2,27	25,00	—	24,27	55,09	95,88	4,12
12.30	26	1,73	20,70	—	21,12	36,54	95,26	4,74
12.45	19	1,27	22,22	—	22,67	28,79	95,59	4,41
13.00	16	1,07	26,32	0,98	26,85	28,73	96,27	3,73
13.30	27	0,90	28,17	—	28,74	25,87	96,52	3,48
14.00	22	0,73	37.50	—	38,26	27,93	97,39	2,61

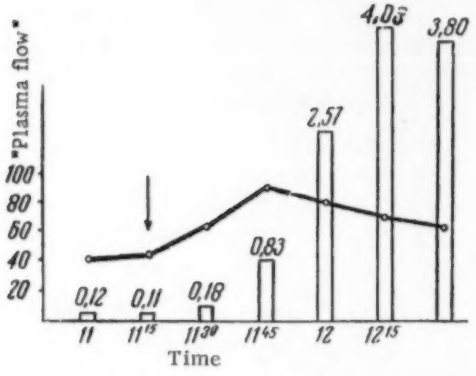

Fig. 2. Effect of a water load on renal circulation and diuresis (dog Rozka, weight 15 kg). Curves — renal "plasma flow" as determined by phenol red, in ml/minute; columns — diuresis in ml/minute; arrow — dog drinks 500 ml of water.

In approximately one third of the experiments, after the water load there was either no change or else an insignificant increase in renal circulation. Thus, there was no direct correspondence between diuresis and renal blood flow.

Consequently, water diuresis after drinking occurs chiefly through reduced reabsorption. It remains to be seen whether the increased diuresis following parenteral injection of fluid is caused by the same mechanism. We have found only one published report [10] dealing with differences in renal function after intravenous injection of isotonic solutions of sodium chloride and glucose: in the first case it appeared that filtration increased while reabsorption remained unchanged, while in the second, on the contrary, the diuresis was caused by a lowered absorption.

The experiments we performed all gave the same results, which are shown in Table 2 and Fig. 3. It can be seen from Table 2 that the increased diuresis in the first 15 minutes after giving fluid intravenously is chiefly due to a greatly increased filtration rate; no great reduction in reabsorption occurred. However, even by the second 15 minute period, the increased diuresis was contributed equally by changes in filtration and in reabsorption. In the third 15 minute period, filtration had returned to its normal value, while reabsorption continued to fall. Subsequently, the high level of diuresis was maintained through reduced reabsorption, while filtration remained within normal limits.

Fig. 3 shows the same result. The initial increase in diuresis depends mostly on filtration, and subsequently it is maintained by reduced absorption. In this experiment, the filtration rate remained raised for a longer period than in the previous one.

Determination of filtration and reabsorption by intravenous injection of a 4.9% glucose solution, showed that the relationship described above is maintained: the initial increase in diuresis is associated chiefly with an increased filtration, while subsequently it is maintained by reduced reabsorption. In this way, we can

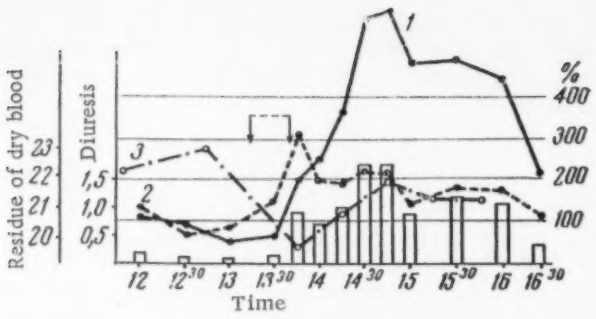

Fig. 3. Effect of an intravenous injection of 400 ml of physiological saline (shown by arrow) on filtration, reabsorption, diuresis, and dry blood residue (dog Kusachka, weight 11 kg). 1) Unabsorbed portion of filtrate, as percentage of original value; 2) filtration (in ml per minute) as a percentage of the original value; 3) dry blood residue as percentage; arrows — dog given 400 ml water; columns — diuresis in ml per minute.

TABLE 3

Effect of Injecting Physiological Saline on Renal Blood Flow (dog Rozka, experiment March 15, 1955)

Time	Volume of urine		Concentration of phenol red (in mg%)		Concentration index	Blood flow (in ml per min)
	total	per minute	in urine	in blood		
12.15—12.30	2,5	0,17	404,0	0,94	429,8	73,07
12.30—12.45	3,2	0,21	481,9	0,97	496,8	104,33
Between 12:43 and 12:57 a.m., 300 ml of 0.85% NaCl solution was given intravenously						
12.45—13.00	29,0	1,93	84,7	0,96	88,2	170,22
13.00—13.15	17,0	1,13	59,5	0,92	64,7	73,11
13.15—13.30	8,0	0,53	133,3	0,88	151,5	80,30

definitely refute the claim referred to above [10] that extra saline is excreted by increased filtration, and glucose by reduced reabsorption.

We have shown that when fluid is injected, filtration plays a more important part in causing the increased diuresis, than when the water is given by mouth. We have yet to explain what causes the increased filtration at the end of the injection. It would seem reasonable to suppose that it is the result of hydremia, which produces an increased filtration pressure. Having observed the hydremic reaction to parenteral fluid injections, we showed that it is more constant than the same reaction in response to food which we demonstrated previously [3]. Immediately after the injection of the fluid, in most experiments a dilution of the blood was observed. However, just as in the experiments in which the water was given by mouth, the degree of hydremia did not correspond to the subsequent course of the diuresis.

If a comparison is made of the change in filtration and reabsorption with change in the dry blood residue (see Fig. 3), then it is easily seen that the time of greatest filtration increase coincides with the maximal dilution of the blood. In cases where no definite hydremia was observed from the very beginning, the increased diuresis was brought about by reduced reabsorption and not by increased filtration.

Thus, there is good reason to suppose that the increased diuresis following intravenous injection of fluid is at first due to an increased filtration, which is brought about by the increased dilution of the blood. Subsequently, the increase is maintained chiefly by reduced reabsorption, while changes in filtration contribute comparatively little , i.e., the condition at this stage is similar to that obtaining when fluid is given by mouth.

Just as when fluid was given by mouth, results of renal blood flow measurements as affected by parenteral injection of fluid were varied. In some, there was no blood flow change, while in most experiments there was an increase. One clear difference was that when giving water by mouth, the maximum increase in blood flow usually occurred 30 minutes after drinking, while when the fluid was injected, the maximum flow always occurred directly at the end of the injection. In most cases the increased blood flow was confined to this period (Table 3); only in one experiment of the twelve was the maximum increase in blood observed after an interval of 30 minutes.

Whether the water was given orally or parenterally, there was no correlation between the changes in blood flow and diuresis..

The work we have done has refined our knowledge of changes in renal filtration and reabsorption when water is introduced by different routes, and has shown that increased blood flow is not an important factor in water diuresis. The reasons for the observed increase in renal circulation during water diuresis and its relationship to filtration rate require further investigation.

SUMMARY

The work was carried out on dogs with ureteric fistulas. They were given 30 mg/ kg of water plus 10% milk to drink; 20-25 ml per kg body weight of isotonic solutions of sodium chloride and glucose were injected intravenously at a rate of 20 ml per minute. Filtration and reabsorption were measured in terms of creatinin clearance, while renal blood flow was determined by injecting an oily suspension of the dye phenol red. Water diuresis occurs after drinking by virtue of a greatly reduced reabsorption. In some cases the diuresis was caused by an increased filtration. Diuresis increased rapidly after intravenous injection of fluid, and this was mainly due at first to increased filtration, but later to diminished reabsorption. The initial increased filtration is accompanied by a pronounced hydremia following the intravenous injection. In the majority of experiments, the renal blood flow was increased during the first 15-30 minutes after drinking, as well as after the intravenous injection.

Later, however, no relation was found between the rates of renal blood flow and diuresis.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

PERIPHERAL BLOOD IN MONKEYS IN ACUTE RADIATION SICKNESS

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It has frequently been demonstrated that one of the most constant and early reactions to ionizing radiation consists of changes in the blood system. Studies of the effect of radiation have been made on most laboratory animals, but very little work has been done on monkeys. Some reports [3-5] have shown that the blood reaction of monkeys bears many resemblances to that of other mammals and of man, and differs only in the degree to which different symptoms are developed.

Because the hemopoietic organs in monkeys in radiation sickness have been but little studied, and because certain problems such as changes in the thrombocytes have not been explored, we thought it worthwhile to investigate changes in peripheral blood in monkeys in response to ionizing radiation.

METHOD

The experiments were carried out on Macacus rhesus monkeys at the Sukhumi Medical and Biological Station. The animals were kept in cages. Their ages ranged from 1½ to 2 years. In all, 18 animals, including 11 males and 7 females, were studied; of these, 13 were irradiated with a dose of 700 r, and 5 served as controls.

The conditions of the experiment were as follows: total irradiation dose 700 r; general field with two tubes, distance 78 cm; maximum voltage on tubes 180 kv, current 15 ma; dose 21.8 r/minute; time of irradiation 32 minutes.

RESULTS

For 3-4 hours after the irradiation, diarrhea and vomiting occurred in most animals; they did not eat, but drank copiously. Subsequently, and until the fifth day, their condition was satisfactory. Physical signs of radiation sickness occurred on the 5-7th days, when the animals spent a considerable part of the day lying down; the normal reflexes were absent, and food remained uneaten. Later, there was bleeding from the nose, and in some cases bloody stools were passed.

In the first few hours after irradiation, in spite of their general satisfactory condition, changes in peripheral blood were observed. Three hours after irradiating, the leukocyte count rose sharply, in some cases to 65,000 to 90,000, while by the end of the first 24 hours it had dropped again to 5,000-8,000. Subsequently, the number of leukocytes became progressively reduced; after 7-12 days, it had fallen to 2000-400 per mm^3 or 10-8 % of the initial value. In one animal only, which has survived this period, the leukocyte count again rose, and by the 30th day had reached a value of 15,000.

Changes in the shape of the leukocytes were also observed a few hours after irradiation. The increased leukocyte count found 3 hours after the beginning of the experiment resulted entirely from an increase in the number of neutrophils, which increased by 3.5 times or more; in some cases there were 60,000-80,000, and on average 39,000 cells per mm^3. The increase in the number of neutrophils coincided with an increase in the number of young forms of these cells (Fig. 1).

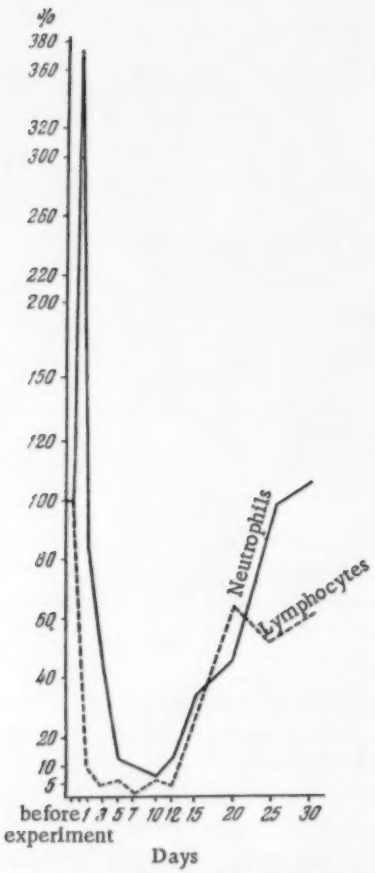

Fig. 1. Changes in average numbers of neutrophils and lymphocytes as a percentage of the original number.

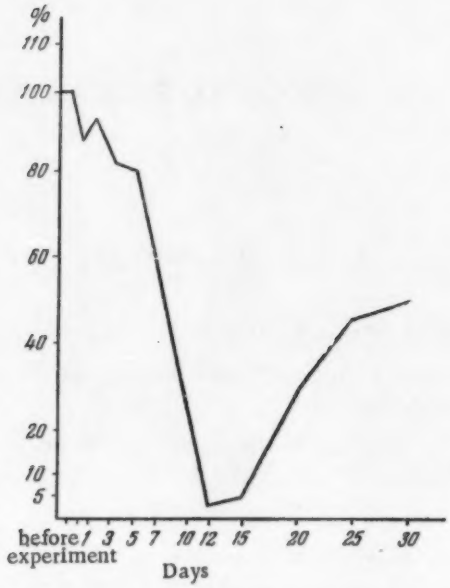

Fig. 2. Changes in thrombocyte count, expressed as a percentage of the original value.

Later, the number of neutrophils fell and reached a minimum value of 1000-700, as compared with an average of 10, 300 cells per mm^3, by the 7-10th day. Subsequent return to normal values was found to occur at the 25-30th day only in the single animal which survived for more than 12 days.

Even as early as 3 hours after the irradiation, the number of lymphocytes was halved: before irradiation it was 5000, and 3 hours later it had fallen to 2500-1500. By the end of the first day, the lymphocyte count was only 10% of the normal (800-400 cells per mm^3) and remained at this level to the end of period of observation.

In the first few days, there was an increase in the number of eosinophils, but later the number fell until only occasional cells could be seen. The changes observed in the monocytes were similar to those in lymphocytes.

On our counts, the average number of thrombocytes was 417,000-474,000. For the first few hours after the irradiation, the number was reduced, and after the 7th day it fell very sharply; by the 10-12th day the minimum value of 20,000-15,000 had been reached. In the single animal which survived to the 31st day, there was some increase after 15 days (Fig. 2).

An increase in the number of young thrombocytes was found 3 hours after irradiating (when there was a fall in the total number of these cells). Subsequently, the number of young and mature platelets became considerably reduced, reaching a minimum value by the 10-12th day. By the 3rd day, there was a marked increase in the number of strongly pyknotic, old thrombocytes, and later these formed the main mass of platelets until the end of the observation period. No thrombocytic irradiation forms were found at any period of the observation (Fig. 3).

The erythrocytes and hemoglobin were much less affected. Whereas no marked changes in the red cells were found, considerable alterations were found in the reticulocytes: 3 hours after irradiation, their number had been halved, and after 5-25 days only a few remained.

In the control group, over the whole period of observation no deviations from the normal blood picture were observed.

From the results presented, it can be seen that as early as 3 hours after the irradiation, the leukocyte count had risen considerably through an increase in the number of neutrophils; the increase was due to the formation of young forms. As has already been pointed out [1, 2, and others], this phenomenon is due to a more rapid maturation of cells in the bone marrow and to an increased rate of entry of these cells into the peripheral blood stream.

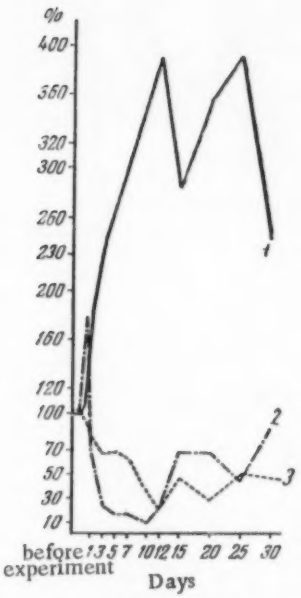

Fig. 3. Changes in the number of thrombocytes as a percentage of the original value. 1) Old thrombocytes; 2) young thrombocytes; 3) mature thrombocytes.

It is well known that lymphocytes are the most vulnerable to radiation, and the considerable reduction in their number found in the first few hours after the treatment evidently results from their increased rate of breakdown.

In the first stage of the reaction, the number of thrombocytes was reduced; however, at this time, owing to an increase in their rate of maturation, the number of young forms in the peripheral blood rose.

It is important to note that no irritation forms were found in any of the irradiated animals; as we have already pointed out, such forms are an unfavorable prognostic sign, and indicate that no regenerative processes are taking place in the megakaryocyte apparatus of the bone marrow.

According to M. Haigh and E. Paterson [5], a progressive reduction in the number of reticulocytes is also a bad prognostic sign in radiation sickness in monkeys. Our investigations confirmed this observation.

From the 3rd. day onwards, there was a reduction of the formed elements of the blood, with the exception of the erythrocytes, whose number was not reduced by more than 20-28% during the whole of the observation period. However, the marked and maintained reduction in the number of reticulocytes shows that the absence of observable changes in the red cells results from the long life of the erythrocytes, taken together with the fact that our observations were not continued for more than 30 days.

In addition to the quantitative changes in the blood cells, qualitative changes were also observed, particularly in the neutrophils and thrombocytes.

The hypersegmentation of the neutrophil nucleus, which is typical of monkey blood, was already well marked by the 3rd day after irradiation. In some of the neutrophils, there were as many as 17-18 segments; occasionally there was a pathological granularity, and a dissociation between the times of maturation of protoplasm and nucleus. The occurrence of pyknotic thrombocytes, the formation of abnormal microforms and pale blue thrombocytes having no granules, and degenerative forms, was an indication that functional changes in the bone marrow had taken place, and that there had been a disturbance in it of hemopoiesis.

SUMMARY

Peripheral blood was studied in 18 monkeys (Macacus rhesus) in acute radiation sickness induced by a single x-ray irradiation with a dose of 600 r.

The principal features of the changes occurring in the blood of these monkeys were, in the main, identical to those revealed in other laboratory animals. In the monkeys, however, typically there were accentuated variations in the number of leukocytes during the first 24 hours, the nuclei formed an abnormally large number of segments, there was a more pronounced microthrombocytosis, and no thrombocytic irritation forms were present.

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RELATION BETWEEN THE SITE OF RADIATION DAMAGE AND THE FUNCTIONAL CONDITION OF THE ORGAN

COMMUNICATION I.

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In connection with pathological, anatomical, and clinical observations, the question as to the relationship between the degree of damage to an organ and the level of its activity has often been discussed [1]. However, until now no experimental investigations have directly confirmed that such a relationship exists. Nevertheless, the problem may well be of considerable theoretical and practical importance, and it is for this reason that the present work has been undertaken.

The disease chosen for study was radiation sickness in which morphological changes occur throughout the body, but their extent varies greatly from one organ to another. Consequently, in this case the attempt could be made to control the extent of the damage by varying the level of activity of the organ by pharmacological means.

The most convenient organs to study were the kidneys, which usually suffer comparatively little damage in acute radiation sickness [2,4]. Damage to the renal parenchyma under appropriate experimental conditions might constitute a convincing demonstration of the truth of the idea under test.

Except for observations made on tissue cultures of the chicken mesonephros [3], we have found no reports of investigations of this kind. In the experiments referred to, secretion of the tubules was induced by adding phenol red or magnesium sulfate to the drinking water. It was found that epithelium which was secreting rapidly was more than usually sensitive to the action of x-rays. These investigations are extremely important, but they do not decide the problem of the localization of the radiation damage in whole organisms under the control of many regulatory systems.

METHOD

We chose diuretics which would exert a selective action on different parts of the nephron. These requirements were most nearly met by theophylline and mersalyl. Theophylline causes a dilatation of the glomerular capillaries, and consequently increases filtration, while mersalyl exerts its principal action on the epithelium of the tubules.

Numerous experiments have been carried out to determine the maximum dose of these drugs which can be tolerated when given intraperitoneally, and the time of their maximum effect. The irradiation was timed to occur at this period.

The experiments were carried out on 400 mice of both sexes weighing 20-25 g, and on 40 guinea pigs weighing 400-450 g, using a double control.

In each experiment, one part of the animals of the same sex and weight was submitted to irradiation only, and the other received only the diuretic. For postmortem examination, the animals were killed by destroying the medulla oblongata.

Irradiation of the whole animals was carried out using an RUM-3 apparatus, used under the following conditions: voltage 180 kv, current 10 ma, filter 0.5 mm Cu and 1 mm Al, intensity 45-48 r/minute, focusing distance 30 cm for mice and 40 cm for guinea pigs, dose 1400 r (measured by a Gris dosimeter). For irradiation, each mouse was placed in separate cardboard box measuring 7 x 4.5 x 4.5 cm, and the experimental and control animals were irradiated simultaneously. Each guinea pigs was irradiated separately in a plywood box measuring 15 x 10 x 9 cm.

RESULTS

All the irradiated animals developed acute radiation sickness. In evaluating the results, attention was paid to the time of survival after irradiation, and to the condition of the renal parenchyma.

Animals irradiated during the period of maximum activity of the diuretic survived for 2-4 days, while controls lived 3-6 days. Consequently, in most cases, increased renal function reduced survival time by about one third.

The symptoms of severe damage were constant in both groups. Usually the fur became disshevelled by the 2-4th day, there was a photophobia, diarrhea, and the animal died 12-24 hours after the onset of clinical symptoms.

Physical signs were few and constant. The animals were emaciated, the stomach was empty, the intestine was distended by fluid and by the gaseous contents, there were sometimes hemorrhages into the intestinal mucosa, liver, lungs, testes and skin. Histological study revealed the typical picture of acute radiation sickness, the only difference between the experimental and the control group being in the condition of the renal parenchyma.

In the first experiment, in which theophylline was given 30-45 minutes before irradiating, there was considerably increased renal damage in 91% of the animals. Because of some variability in the times at which the animals died, it was possible to follow the changes occurring in the kidneys. At first there was a marked and frequently uneven engorgement of the glomeruli with blood. They increased in size, and the vascular plexus lost its usual clear outline.

Somewhat later there was a change in the structure of the vessels walls, they became swollen, lost their structure, and became basophil. The endothelial cells began to necrose, and most of the nuclei showed a clear central zone and an accumulation of large grains of chromatin beneath the membrane. The number of nuclei in a glomerulus became reduced from the normal 50-60 per section 10-12 μ thick, to 12-15.

The lumen of the blood vessels was lost through the great increase in the thickness of the walls, and many glomeruli lost their structure (Fig. 1). Sometimes the cavity of Bowmans capsule was enlarged and filled with an amorphous oxyphil mass. In animals which survived longer, necrosis of some of the loops of the glomeruli occurred, together with thrumbosis of some of the vessels. The glomeruli were considerably enlarged, and the outline of the vascular plexus was lost in an amorphous mass of strongly basophil thrombi .

There were numerous hemorrhages around the glomeruli. Necrosis of the arterial walls followed by formation of thrombi was found in the stroma of the renal cortex. Other glomeruli, usually fewer in number, preserved their normal structure. In the epithelium of the main portions of the tubules, varying degrees of dystrophy could be seen. Necrotic changes were common in the tubules when there was extensive thrombosis of the vessels.

In the second experiment, in which mersalyl was injected 45-90 minutes before irradiating, renal damage occurred in 86 % of the animals. The renal parenchyma exhibited a characteristic change. The glomeruli retained their normal structure, and only in places could some swelling and loss of structure, be observed together with basophilia of the vessel walls and necrosis of individual endothelial cells. In some cases there was vascular engorgement of separate loops of the glomeruli. In the stroma, zones were found where the arterioles had necrosed, and the walls had lost their structure.

The main portions of the tubules were greatly thickened through a swelling of the epithelium, which almost filled the lumen. Many of the cells had indistinct outlines and a granular cytoplasm, and there were

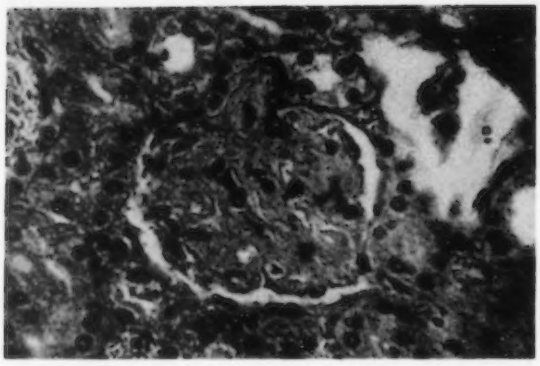

Fig. 1. Renal changes following the injection of theophylline and general radiation. Swelling and loss of structure of glomerular vessel walls, disappearance of nuclei. (Van Gieson's stain. Magnification, eyepiece 10 x, objective 60 x).

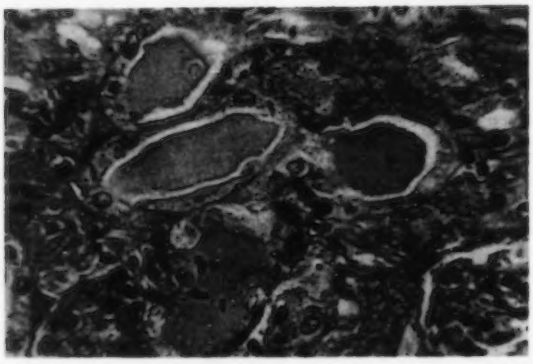

Fig. 2. Renal changes following injection of mersalyl and radiation. Cylinders staining with picric acid present in lumen of canals. (Same stain. Magnification eyepiece 12 x, objective 45 x).

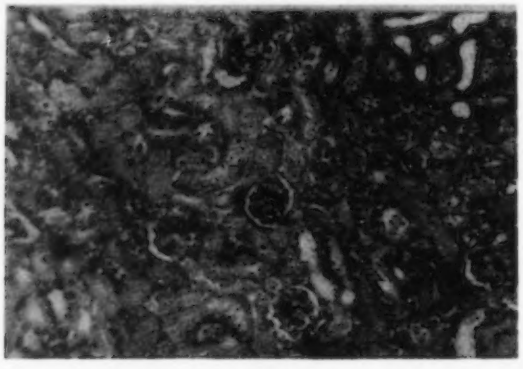

Fig. 3. Changes in the glomeruli and tubules following injections of mersalyl and exposing the whole animal to radiation. Glomeruli intact, epithelium of the main portions of the canal is necrotic. Same stain, eyepiece 10x objective 20x .

no nuclei, these having broken up into an amorphous mass, or into separate clumps. In many cases the visceral layer of the capsule was considerably swollen, and many of the cells had died.

By use of various methods and stains (polarization microscopy, use of osmium, Sudan, neutral red, Nile blue) it has been shown that in the dying epithelial cells and in the mass of dead cell remnants which fills the lumen of the tubules, there is a small amount of fat, most of it neutral. The main portion of the detritus consists of both small and large droplets, staining dark with osmium and yellow-brown with Sudan. With Van Gieson's stain, the cytoplasm of the epithelium reveals small uniform masses which stain deeply with picric acid. In the necrotic areas, these inclusions are larger and present in greater numbers, and in parts they fill the lumen of the canals, and finally form numerous cylinders (Fig. 2).

In the control groups of both experiments, which received only the radiation, the renal damage was restricted to necrosis of isolated cells of the endothelium of the glomeruli; there was a very slight swelling of the vessel walls and very slight degenerative changes of the epithelium of the tubules. In another group of control animals, which were given diuretics only, and which were used for determining the dose, no morphological changes occurred in the renal parenchyma. Thus, the trend of the results indicates clearly that the shortness of the survival time of the experimental animals results from radiation damage to the renal parenchyma, leading to functional disturbances.

The most important result was that which showed the influence of diuretics on the extent of the renal damage, and its dependence on the time of their injection. Thus, when theophylline was used, the maximum change in the renal parenchyma was observed when it was injected 30-45 minutes before irradiation. If the period of irradiation was extended up to 60-90 minutes, renal damage was considerably reduced. Finally, if the irradiation was given 15 minutes after the theophylline injection, the kidneys were not affected.

Mersalyl was effective in increasing the severity of the radiation sickness only when the irradiation was given less than 45 minutes after the injection, and the maximum effect was observed after 60-90 minutes, but was sometimes maintained for longer periods. The period of maximum effectiveness with respect to increasing the radiation damage of the renal parenchyma coincided exactly with the period of maximum diuretic action, as

determined in preliminary experiments. The results afford a clear illustration of the critical importance of the functional condition of the nephron during irradiation.

It now becomes necessary to explain the reason for the failure in 11.5% of the experiments, when no renal damage followed irradiation, and in 8% of the experiments when the injection of diuretics produced no effect, or in the 3.5 % of the animals where the changes in the nephron were better shown in the control animals. These results are probably due to giving the irradiation when the kidneys were not functioning at their maximum efficiency, or, in other cases, due to irradiating the control animals at a time when the kidneys happened to be particularly active.

Microscopical investigation demonstrated well-marked radiational renal damage in the animals which received injections of diuretic substances. The site of maximal change in the neurone depended to a remarkable extent on the particular diuretic used. Thus, theophylline causes, principally, damage to the vascular portion of the nephron. With mersalyl, however, these is a striking difference between the main portions of the tubules, which necrose, and the glomeruli, which retain their structure (Fig. 3).

Detailed studies of radiational damage to the epithelial portion of the nephron have demonstrated characteristic features. It appears that the damage to the tissues leads to the formation of harmful lipoprotein complexes giving an unusual reaction with Sudan (pale yellow-grey color). Possibly, this particular kind of necrosis is confined to radiational damage.

In the experiments described, it may be assumed that the site of the damage was determined by the functional condition of the kidney at the time of irradiation.

SUMMARY

A study was made of the relationship between local radiational damage and the functional condition of the kidney. Kidneys were chosen for investigation, because they usually do not suffer much in radiation sickness. Function was stimulated pharmacologically.

The experiments were performed on 400 mice and 40 guinea pigs. Total irradiation was timed to coincide with the period of maximal effect of the diuretics. An RUM-3 apparatus was used, at 180 kv, 10 ma, filter 0.5 mm Cu and 1 mm Al, focal length 30 cm for mice and 40 cm for guinea pigs, dose 1400 r.

When the kidneys are stimulated to function at an abnormally high rate, the result is to confine the radiation damage to the kidney parenchyma. When theophylline is given, the vascular portion of the nephron is affected, and with mersalyl it is the epithelial region which is damaged; the effect obtained depends on the diuretic mechanism.

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CHANGE IN CHOLINESTERASE ACTIVITY IN ACUTE RADIATION SICKNESS

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The view that radiation sickness results from a failure of coordination of the different organs and systems makes it necessary to examine, carefully, disturbances of the basic regulatory mechanisms occurring in this disease. First in importance are the enzyme systems, and in particular cholinesterase, the enzyme responsible for mediating central nervous excitation and its transmission to effector organs.

In the present work, an attempt has been made to study cholinesterase activity in several different tissues.

METHOD

The experiments were carried out on mice and on frogs. The mice were exposed to gamma radiation from radioactive mesothorium, the dose being 700 or 10 000 r ± 10%. When irradiated with 700 r, all the mice died within a month. After irradiating with 10,000 r, all the animals died within 3-4 days. Cholinesterase activity measurements were made in the tissues of the brain, liver, and intestine, and in addition, the sensitivity of the irradiated mice to strychnine was measured.

In experiments on frogs, an ampoule containing 100 mC units of mesothorium (lead filter 1 mm) was sewn in position over a vertebra. The irradiation was continued for 3-5 days. On the 14-16 day, an abscess had formed at the site of the ampoule. The animals died after 2-3 weeks. Convulsions occurred one week after the irradiation. In frogs, measurements were made of the cholinesterase activity in the spinal cord, and changes in the sensitivity to strychnine and neostigmine were noted. Strychnine was given intraperitoneally to mice as a 1: 50,000 and to frogs as a 1: 10,000 solution, the dose being the same per g body weight. Neostigmine diluted 1: 1000 was injected intraperitoneally into frogs.

Cholinesterase activity was measured using R. Ammon's method [2] and a Warburg's apparatus.

RESULTS

Measurements of cholinesterase activity. Table 1 shows results of measurements on the spinal cord of irradiated and control frogs.

From Table 1 it can be seen that at the end of the irradiation, there is an immediate drop in cholinesterase activity of 35% as compared with the controls. It continues to fall, and after 2 days has reached 40% of the control value, i.e., it has been reduced by 60%.

Table 2 shows the results of corresponding measurements in control mice.

It can be seen that the greatest activity is found in the liver, and the least in the brain. The activity is more constant in the latter.

Table 3 shows the results of measurements of cholinesterase activity in mouse tissue after irradiation with 10 000 r. These results must be considered as appoximate, on account of the small number of experiments and the comparatively wide scatter in the results obtained. However, a comparison is shown between these results and those of Table 2, and it can be seen that after irradiation, there is a reduction in cholinesterase

TABLE 1

Cholinesterase Activity in γ of Hydrolyzed Acetylcholine per 100 mg Spinal Cord per 40 Minutes

No. of animal	Control	Immediately after irrad.	Two days after irradiation
1	2 057	2 43	1 050
2	1 940	2 360	1 740
3	2 200	1 770	1 700
4	2 070	2 170	1 050
5	2 070	1 323	1 220
6	1 740	1 270	540
7	2 800	1 280	1 100
8	2 600	1 803	870
9	2 600	—	—
10	2 700	—	—
Average	2 277	1 482	927

TABLE 2

Cholinesterase Activity in γ of Hydrolyzed Acetylcholine per 100 mg Tissue per 40 Minutes

No. of animal	Liver	Brain	Intestine
1	2 426	512	1 070
2	1 552	627	1 326
3	2 820	672	1 443
4	2 692	588	1 364
5	2 840	617	723
6	3 070	672	987
7	2 046	578	1 435
8	2 112	526	1 048
9	2 334	515	946
10	2 198	613	947
Average	2 410	592	1 129

TABLE 3

Cholinesterase Activity, in γ of Hydrolyzed Acetylcholine per 100 mg of Tissue per 40 Minutes

No. of experiment	Liver				Brain				Intestine			
	Time (in days) after irradiation				Time (in days) after irradiation				Time (in days) after irradiation			
	1	2	3	4	1	2	3	4	1	2	3	4
1	1 260	2 088	—	—	752	1 265	—	—	936	1 315	—	—
2	1 476	1 882	2 838	2 532	391	534	312	259	1 132	923	1 491	880
3	1 468	2 266	2 038	1 194	245	347	253	300	1 157	—	1 034	775
Average	1 403	2 078	2 438	1 863	492	715	282	279	1 075	1 119	1 262	817

activity. For the liver, this reduction was greatest on the first and fourth days after irradiation, being 30 % on the first day and 20% on the fourth, while for the brain the corresponding figures were 17% and 50%; for the intestine, there was a 25% reduction by the fourth day.

Table 4 shows the results of cholinesterase activity measurements in mice irradiated with 700 r.

These results show that this dose causes an appreciable change. The reduction is on average:

Organ	First Week	Second Week	Third Week
Liver	22 %		23 %
Brain	28 %	14 %	
Intestine	19 %		24 %

The effect of strychnine and neostigmine on convulsions in irradiated animals. Frogs in which the cord was irradiated showed an increased sensitivity to strychnine and to neostigmine. In the control group of 50 animals, convulsions occurred 20 minutes after the injection of 2γ per g of strychnine, or 10γ per g of neostigmine.

The spinal cord was irradiated in 40 frogs for 3 days; 1 per g of strychnine was injected into 20 of these and 5γ per g into the remaining 20, and in each case convulsion occurred after 20 minutes.

TABLE 4

Cholinesterase Activity, in γ of Hydrolyzed Acetylcholine per 100 mg of Tissue per 40 Minutes

No. of experiment	Liver			Brain			Intestine		
	Time (in weeks) after irradiation			Time (in weeks) after irradiation			Time (in weeks) after irradiation		
	1	2	3	1	2	3	1	2	3
1	1 226	3 102	1 470	430	—	464	808	1 106	845
2	1 464	2 304	1 836	304	540	595	923	1 198	805
3	2 862	2 580	2 186	441	390	403	945	1 026	607
4	2 000	2 204	2 052	461	540	691	784	1 201	1 132
5	—	2 008	1 770	523	631	476	1 176	1 022	918
Average	1 880	2 430	1 860	432	520	525	922	1 110	861

No increased sensitivity to strychnine was found to follow irradiation in mice. In the control group, the convulsions occurred 20 minutes after injecting 0.08 ml of a 1: 50,000 solution per g weight of strychnine (10 animals); 0.05 and 0.07 ml of the same solution caused only an increased excitability over the same period (20 animals used). After irradiating with 700 r, an injection of 0.05 ml of strychnine per g again caused only a small increase in excitability for the first 4 weeks after irradiation (50 animals). The same result was observed during the first 3 days after irradiating 30 frogs with 10,000 r. It was interesting that right up to the time of death, there was no increased sensitivity to strychnine.

From our experiments it follows that for both frogs and mice, the activity of cholinesterase is reduced after irradiation. The greatest fall in activity in mice corresponds with the period of the primary reaction and the time of manifestation of acute radiation sickness. The great reduction in the cholinesterase activity in frogs as compared with mice may be due to the much greater dose of radiation. There is no doubt that cholinesterase activity in tissues is reduced after irradiation, and confirmation has been given in other published reports.

Burn, Kordik, and Mole [3] found a reduced pseudocholinesterase activity, and Conard [4] found a reduced cholinesterase activity in the intestine of a rat receiving a total radiation dose of 500-1000 r. Nachmansohn [5] attributes changes in the acetylcholine — cholinesterase system to the effect of the ionizing radiation on the nervous conducting elements.

The experiments on the development of convulsions in mice following strychnine injections, showed that irradiation did not raise the sensitivity to this drug. However, there was an increased sensitivity to strychnine in the same experiment in frogs. Possibly, the difference may depend on the dose, and on the region irradiated, which, in frogs, was the spinal cord. Also, in frogs, radiation by itself may induce spasms, and strychnine merely facilitates their development, while in warm-blooded animals not many authors have observed definite convulsions, and these occurred only just before death from very large doses.

SUMMARY

Total gamma irradiation of mice with a dose of 700-1000 r provoked a decrease of cholinesterase activity in brain, liver and intestine; this occurred during the first and third week after irradiation, during the period of the primary reaction when the disease was at its height; the change was especially pronounced shortly before death.

Total gamma irradiation with 700-10,000 r causes an increased sensitivity to strychnine in frogs, but not in mice.

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THE EFFECT OF TOTAL X-RAY IRRADIATION ON THE THALAMUS

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The object of the present work has been to study the functional condition of sympathetic centers in the hypothalamus during acute radiation sickness, and also to determine thresholds for hypothalamic effects produced by ionizing radiation.

Central autonomic regulatory function is of great importance in connection with the extensive evidence of destruction of the innervation of endocrine glands by radiation. The quantity measured was the change in the potentials developed by the sweat-secreting surface of the cat's paw in response to hypothalamic electrical stimulation. It is known that this reaction is mediated nervously, and that the hypothalamic excitation is conducted to the effector organ (the sweat gland) by sympathetic cholinergic fibers.

METHOD

The experiments were carried out on 20 cats, 12 of which received a dose of 600 r and 4 a dose of 50 r, while the remaining 4 served as controls. The electrodes were implanted in the hypothalamic region using Hess's method. Postmortem observations on the position of the tips of the electrodes showed that they lay in the anteromedial hypothalamic areas (mainly in the nucleus supraopticus, nucleus medialis, and nucleus lateralis).

Stimulation was applied to the hypothalamic region with a sinusoidal voltage from a ZG-10 oscillator at 50 cycles per seconds at intervals of 2-3 minutes. The strength of the stimulus varied according to the threshold, and varied from 0.2 to 3.6 volts.

The sweat secretion was recorded by the method of Weng and Brown, as used for measuring the cutaneous-galvanic reflex.

Change in skin potential, and respiration, were recorded from the ensuing reaction after the end of the stimulation on a two-channel electrocardiograph (mingograph 24 Elema). A feature of the apparatus was the inertia-free inkwriter. The paper speed was 0.25 cm/second. The magnitude of the reaction was expressed in millivolts.

The electrodes were clips of chemically pure sheet zinc firmly fixed to the front paw: one electrode was applied to a pad of the foot (sweat-secreting surface), and the other to the shaved dorsal surface. A layer of gauze soaked in a paste consisting of a mixture of kaolin and saturated zinc sulfate solution was placed between the skin and electrode. All the experiments were carried out under ether anesthesia.

RESULTS

A preliminary study was made of the sweat-secreting reaction in response to hypothalamic stimulation in normal cats. The baseline curve in anesthetized animals was usually straight. Sometimes potential variations not exceeding 0.3-0.4 mv were observed. Isolated spontaneous waves occurred frequently.

The threshold value of the stimulus required to elicit a change in potential from the sweat glands varied in different animals by as much as from 0.2 to 2.5-3 volts (see table).

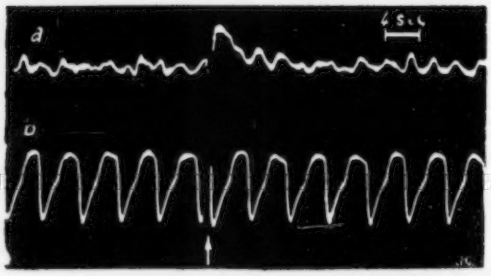

Fig. 1. Change in (a) potential difference of sweat glands, and (b) respiration, on stimulating hypothalamus (control). Arrow (↑) indicates moment of stimulation (0.2 volt, 30 seconds).

Variation in Successive Experiments of Hypothalamic Stimulus Threshold in Control Animals (results given in volts, interval between experiments 2-3 days)

No. of cat	Expt. No. 1	Expt. No. 2	Expt. No. 3
22	0.2	0.2	0.2
29	0.2	0.2	0.2
34	1.3	1.5	1.5
36	1.0	1.3	1.3

The sweat-secretion reaction in response to hypothalamic stimulation developed gradually, and it was always possible to record it after the end of the stimulation. It was very rare for the potentials to begin as early as 10-15 seconds after the onset of stimulation, but they usually occurred after the stimulus had ceased. The potential change was always in the same direction. The magnitude of the effect obtained was 0.6-1 mv, and was not always a function of the strength of the stimulus.

As a rule, the respiration after the stimulus had been given did not differ from the normal condition. In some experiments, when very strong stimuli were used, it was slowed, and respiratory movements might even cease.

Fig. 1 shows the baseline curve, as well as the reaction of the sweat glands to the hypothalamic stimulus.

After preliminary results had been obtained, 12 cats were exposed to a dose of 600 r, administered as follows: whole animal irradiated, three-tube apparatus potential 180 kv, current 20 ma, focusing distance 60 cm, dose rate 22.2-24.9 r/minute, filters 0.5 mm Cu + 1 mm Al.

One experiment on each cat was performed before irradiation. After it, the first test was made after 1-3 hours or after 24 hours, and subsequently after 3 and after 6-7 days. In all, 3-4 experiments per animal were performed.

Irradiation with 600 r caused acute radiation sickness in all the animals. After 3 days, there was generally a severe leukopenia, with a white cell count of 2500-600 cells per mm^3, and there was a noticeable loss of weight.

Death occurred from radiation sickness when practically no leukocytes remained in the blood stream (150-250 cells per mm^3), and after considerable loss in weight had occurred. Of the 12 cats, 3 died within the first 3 days, 3 after 4-5 days, and 5 after 7-10 days. One died under anesthesia 3 days after irradiation.

In the irradiated animals there was a definite phasic disturbance of the functional condition of the hypothalamic region; this took the form of variation in the threshold stimulus required to obtain the reaction of the sweat glands. There was no change in the spontaneous fluctuations recorded from the sweat glands, nor in the type or magnitude of the reaction itself.

Fig. 2 shows separately the results for animals with initially high and low hypothalamic stimulation thresholds (low and high excitability respectively).

Of the 12 cats irradiated with 600 r, in 7, before irradiation, the threshold varied between 1.5 and 3 volts, and in 5, from 0.2-0.5 volt; in all these animals, before irradiation, application of these stimuli to the hypothalamus caused a very definite electrical change in the sweat glands.

In Fig. 2, it can be seen that in the first few hours after the irradiation, there is a considerable increase in the excitability of the hypothalamic centers. At this period, experiments were carried out on 9 cats. In 6 of them there was a marked reduction in the threshold (from 2-2.5 to 0.2-0.5 volts). In one animal there was no reaction from the sweat glands, and in 2 which had low initial thresholds no change was recorded.

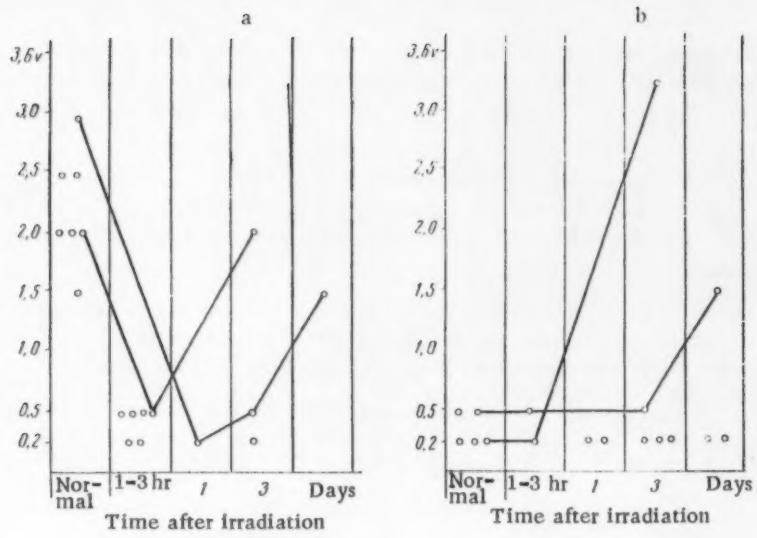

Fig. 2. Variation in hypothalamic stimulus threshold following irradiation of cats with (a) a high initial threshold and (b) a low initial threshold, with 600 r.

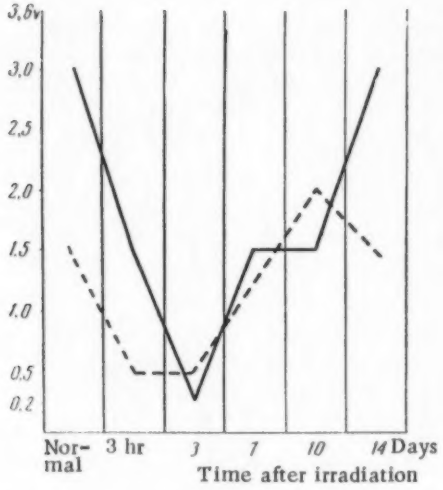

Fig. 3. Changes in threshold of excitability of the hypothalamus after irradiating with a dose of 50 r.

One day after irradiating, in 2 out of 3 animals tested, the threshold remained at its initial value (0.2 volt), while in one the threshold was considerably below normal.

After 3 days, in 7 out of the 9 animals tested, the excitability was raised (in 2 of these, the thresholds were low even before irradiation). In the 2 others (Nos. 7 and 41) there was a marked fall in excitability. In cat No. 7 it had fallen to the initial threshold value of 2 volts, while in No. 41, the threshold before irradiation was low, having a value of 0.2 volt, and 3 days after it had risen to over 3 volts. Both animals died on the following day, i.e., 4 days after irradiation. After 6-7 days, 4 experiments only were carried out, since most of the animals had died by this time. In 2 of these, which had a high initial hypothalamic excitability, no change was observed. In one animal the threshold had fallen from 3 volts before irradiation to 0.2-0.5 volt on the 1-3rd day, and to 1.5 volts on the 6th day. In cat No. 10 there was a marked increase in threshold from 0.5 v before irradiation to 1.5 v on the 6th day; one day later the animal died.

Thus, immediately after irradiation, and also one day later, the excitability of the sympathetic hypothalamic area increased sharply, as was shown by the reduction in threshold stimulus strength; the same thing was true of most animals after 3 days. Just before death, there is a reduction in hypothalamic excitability, which, in animals having naturally high values, returns either to its initial value or falls considerably below it. Thus, two phases of the change of functional condition of the hypothalamic region can be distinguished in acute radiation sickness. At first it functions more vigorously, but toward the end the level of activity is reduced. These differences can be more clearly seen from the curves plotted for two different animals (Fig. 2, a and b). In some animals, with initially high hypothalamic excitability, no change occurred.

Experiments using a small dose (50 r) were made on 4 cats having initially high thresholds (1.3 to 3 volts), i.e., a low level of excitability. Total irradiation was carried out on a single tube apparatus, under conditions as follows: potential 180 kv, current 15 ma, focusing distance 120 cm, dose rate 4 r/minute, filters 0.5 mm Cu + 1 mm Al.

This irradiation dose caused no visible radiation sickness symptoms. During the whole two-week period of observation, the animals did not lose weight, and there was no change in the number of leukocytes in the blood stream. However, in two of the four animals there was a marked change in the functional condition of the higher automatic centers.

Threshold measurements were made after 2-3 hours, and after 3, 7, 10, and 14 days from the time of irradiation. Excitability changes in the hypothalamic area appeared at the same time and in the same direction as in experiments in which fatal doses were given. The results of this experiment on two cats are shown in Fig. 3.

Just as when irradiating with a dose of 600 r, there was a biphasic change in the fuctional condition of the hypothalamic area. In the first 3 days after the irradiation, excitability was increased. After 10 days, it was reduced. Subsequently, the thresholds returned to their initial values.

Thus, the hypothalamic sympathetic centers may be very sensitive to the action of ionizing radiation.

Some results obtained by A. L. Shabadash [6] are very interesting in this connection; using a histochemical method, he demonstrated disturbances of metabolism in cells of the diencephalon following total irradiation with 25-100 r of x-rays.

The results obtained demonstrate definitely the occurrence of a functional disturbance of the hypothalamic sympathetic areas following total irradiation. When these results are compared with the many other published observations, it can be seen that the part played by the hypothalamic area in the reaction of the organism to harmful radiations is complex. The effects appear soon after the radiation, and result in changes of excitability, in increased electrical activity [1, 2, 4], and also in the destruction of the neurosecretory regulatory mechanism [3, 5].

SUMMARY

A study was made of the functional condition of the hypothalamic area in cats following a total x-ray irradiation dose of 600 r. The functional condition of the sympathetic hypothalamic centers was measured in terms of the change in the skin potential of the sweat secreting surface of the cat's paw following electrical stimulation of the hypothalamus.

The excitability of the hypothalamic centers rose considerably after irradiation. The increased excitability was still present during the following 24 hours, and in most cases even after 3 days. Just before death, the excitability of the autonomic centers was reduced.

Analogous phasic changes of hypothalamic excitability were observed in a number of animals following their total irradiation with a dose of 50 r; these disappeared within 2 weeks after irradiation, and no radiation sickness developed.

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SUBCORTICAL NEUROSIS

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The present report represents one section of a series of investigations begun in 1954; in these, a study was made of the leukocytic reaction following the injection of various doses of sodium nucleate into animals suffering from radiation sickness and into other intact or decorticate animals in which neurosis had been induced experimentally. During the course of these investigations, we were able to examine 25 decorticate rabbits, including 7 suffering from a pathological condition of the subcortical structures, which we have named "subcortical neurosis".

When the experiment started, we had found no references in the literature to what happens when different kinds of unconditioned reflexes oppose each other in decorticate animals. Recently, we have been able to compare some of our results with those of A. S. Chechulin [1]; he experimented on two decorticate cats, and observed the breakdown of many autonomic functions after repeatedly opposing the defensive with the feeding reflex.

METHOD

Both hemispheres were removed at the same time. Subsequent examination of the brain showed that the removal had been sufficiently complete. In control animals, the skull was trepined, the dura mater cut, and then sewn up.

Unlike decorticate preparations observed by other investigators (for instance, N. Yu. Belenkov's cats), in our animals, no feeding reflexes were developed: the rabbits could not find food for themselves on the floor of the cage, or make use of it. They were kept alive exclusively by feeding with flour mixed with milk and shredded beet root placed in the mouth. In the first few days after the operation, every attempt to introduce the milk and flour mixture into the mouth of the decorticate rabbits caused a violent defensive motor reaction. Quite soon, after 4-5-6 days, the defensive reaction became weaker and lasted for a shorter time, though it was never replaced by a feeding reaction. It is scarcely possible to interpret this result as representing any primitive conditioned reflex. We appear here to be concerned rather with the phenomenon of "bahnung" and with facilitating a change to physiological dominants. It is interesting that even a short-lasting disturbance to the gastrointestinal tract, such as dyspepsia for a day, did not disturb tha balance of the dominants in favor of preponderance of the defensive reaction. On the contrary, injecting aminasine 2-3 days after removal of the hemispheres, immediately facilitated the replacement of the defensive by the feeding dominant.

A pathological condition was induced in the higher nervous centers by arranging for a conflict to occur between the unconditioned feeding and defensive reflexes; this was carried out 2-3 times per day for 2 days. The same method of opposing the unconditioned reflexes was used for both the intact and the operated animals, in the following way. While feeding the rabbit on the flour and milk mixture, a voltage sufficient to produce a motor defensive reflex was applied to the metal end of the syringe; the voltage was gradually raised until the motor response occurred. In control experiments, decorticate animals were given 6 applications in 2 days of a voltage of the same strength, but without feeding. Electrical stimulation given 1 hour before feeding did not alter the placement of the defensive by the feeding reaction either on the day which it was given, or subsequently.

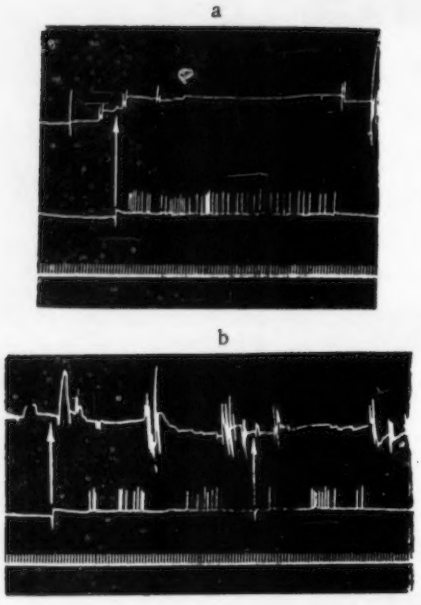

Fig. 1. Formation of an abnormally strong defensive dominant in Rabbit No. 83. a) On the day of "confusion"; b) on the fifth day after confusion. Curves, from above downwards: motor reaction, swallowing movements time marker (5 seconds), ↑ , placing syringe in the mouth .

The conflict between the two different unconditioned reflexes was introduced 2 weeks after decortication. The time appeared optimal because , on the one hand, at this period the direct aftereffects of the operation have disappeared and on the other, compensatory changes induced by the loss of cortex have not yet appeared, nor have degenerative processes in the subcortical structures yet taken place.

Naturally, in order to determine the functional condition of the subcortical cerebral structures which develops as a result of opposing the unconditioned reflexes, it was important first of all to determine what changes occur in the feeding and defensive reactions directly involved in the conflict. It appeared that on the day of the "conflict" and one hour after it, there was no disturbance of the defensive dominant; for this reason we cannot interpret the phenomena which will be discussed below as being due to the laboration of a conditioned defensive reflex. Only 3-5 days after the "conflict" of the reflexes (an not immediately!) did an over-developed defensive dominant develop: the animals hurled themselves about the cage, struggled free from being held, and it was extremely difficult to feed them at this period (rabbits No. 54, 73, 74, 83, etc.).

Much less frequently (rabbits No. 57, 58, and 85) we found that the feeding dominant was stable: the rabbit continued to "chew the air" , and chewed inedible objects such as cottonwool, if they were placed in the mouth; even pricking caused no defensive reaction, but increased the strength of the chewing movements. It was noted that the preponderance of the feeding dominant was found in Rabbit No. 57 before "conflict" of the unconditioned reflexes. Only the comparatively strong pain stimulation during feeding caused this animal to change over from the feeding to the defensive reaction. We may suppose, therefore, that the changeover of the unconditioned reflex activity toward a stable defensive or feeding dominant is to some extent determined by the relative strength of these dominants before the "conflict" .

The formation of an abnormally strong defensive dominant is illustrated by the kymograms for rabbit No. 83 (Fig. 1, a and b). A stable feeding reaction developed on the 4-5th day after the operation. Three electrical stimulations given on the 8-9 day did not interfere with the feeding reaction when the animal was fed one hour after stimulation; the same result occurred on subsequent days. The feeding reaction remained unchanged even on the days of the "conflict" (see Fig. 1, a). However, a marked change occurred subsequently: when the syringe was put into the mouth , the animal developed a desperate defensive reaction, swallowing was continually interrupted, and the defensive reaction dominated the situation (see Fig. 1, b). During this period, and sometimes later, there was a disturbance of autonomic function: the animals lost weight, and the fur was shed; there was a gastrointestinal upset, and postmortem examination of rabbit No. 73 showed erosion of the gastric mucosa.

A careful study was made of the leukocytic reactions (LR) to the injection of small and large doses of sodium nucleate (NaN). A subcutaneous injection of 1 or 3 ml of a 5% solution of NaN was given into the back. Blood from an ear vein was taken immediately before the injection, and 1, 3, and 6 hours afterwards, and a further sample was taken the next day. The interval between successive injections into one animal was, as a rule, 1-3 days. If the number of leukocytes found 1, 3 and 6 hours after the NaN injection even once exceeded the initial level, the reaction was taken as positive; if in all three test the leukocyte count was below normal, the reaction was ranked as inhibitory. Variations of less than 1000 in the leukocyte count were ignored.

The results of investigating the LR in rabbit No. 74 are shown in Fig. 2.

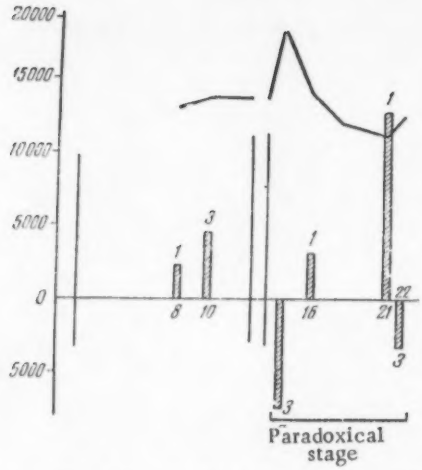

Fig. 2. Change in the leukocytic reaction in rabbit No. 74 induced by injecting 5% solution of sodium nucleate. ——— Initial level of leukocytes on days of experiments Columns show the difference between the maximal increase or decrease in the number of leukocytes after injecting sodium nucleate and the initial value. The figures over the columns indicate the volume in ml of injected solution. The scale shows the numbers along the horizontal axis represent the time, in days, after the decortication.

Here it can be seen that extirpation of the hemispheres does not by itself disturb the normal strength relationships. After "conflict", a paradoxical stage with reversal of the LR occurred: small doses of NaN (1 ml) caused a marked increase in the number of leukocytes from 2500 to 14,000, while large doses (3 ml), on the other hand, actually reduced the number by 2500-6000.

Stimulation with a current of the same strength without feeding, caused no reversal of the LR. After the "confusion" the LR was inverted as in the narcotic, equalizing, paradoxical and inhibitory stage. Also, in intact and decorticate animals with experimental neurosis, we observed particular kinds of distortion of the LR consisting of disturbances of the strength relationships concerned in inhibitory reactions (reactions in which the number of leukocytes was reduced), whereas a large dose of NaN reduced the number of leukocytes to the same extent or even less than did a large dose (equalizing-inhibitory and paradoxical-inhibitory stages). We never once encountered these stages in radiation sickness, because in this disease, the inhibitory stage shows normal strength relationships right down "to zero": the greater the dose of NaN, the greater is the fall in the number of leukocytes.

Total exposure of rabbits with subcortical neurosis to a dose of 400 r (RUM-3; 180 kv; 15 ma; F— 50 cm; Cu 0.5 + 1 Al; 20.3 r/minute) induced a particularly acute form of radiation sickness, and the animals died after 4-5 days (rabbits No. 57 and 58). After exposure to a total irradiation dose of 400 r, decorticate animals which had not been "confused" did not die until the 11-17th day, while control irradiated rabbits recovered.

It remains to enquire in what way subcortical neurosis in decorticate rabbits differs from the corresponding condition in intact animals. First, in intact animals, we never succeeded in observing the over-development of the defensive and feeding dominants after "conflict". In these animals, the conditioned defensive reaction which arose became rapidly transformed into the usual feeding reaction. The effects of "conflict" in intact animals are shown chiefly in autonomic disturbances, and not in their "everyday" behavior. Secondly, conflicts between the defensive and feeding reflexes in the decorticate animal lead to reversal of the LR immediately after the "conflict". In intact animals, the greatest autonomic LR disturbance develops by the 5-8th day after the "confusion".

It is very intesresting that in five rabbits which had recovered from experimental neurosis, and in which the change in the LR to increased amounts of NaN had become quite normal, decortication produced a second reversal of the LR. Decortication of rabbits which had recovered from radiation sickness does not cause this inversion, if the normal relationship of the LR to strength of dose has been reestablished.

Our conclusion is that the experiments demonstrate the important part played by subcortical structures in the pathogenesis of neurosis. The severity of the condition in decorticate as compared with intact animals, and the return after decortication of a neurosis from which the animal had previously recovered demonstrate the important part played by the cortex in compensation of function lost as a result of the neurosis. In this respect, our findings and conclusions agree with those of A. S. Chechulin.

SUMMARY

Subcortical neuroses, which is a condition affecting subcortial structures, was induced in decorticate rabbits by producing a conflict between the food and defensive unconditioned reflexes. A stable dominant, usually of the defensive type, is developed, and there is a disturbance of autonomic function with a reversal of the leukocytic reaction induced by injecting various doses of sodium nucleate. Decortication of rabbits which

had recovered from an experimentally induced neurosis of this type caused a second reversal of the leukocytic reaction.

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MECHANISM OF INSULIN COMA ARREST BY INTRAVENOUS GLUCOSE INJECTIONS

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Injection of 40 % glucose solution is the recognized method of treatment for insulin coma. In recent years it has been shown that glucose may be replaced by hypertonic solutions of inorganic substances such as sodium chloride, magnesium sulfate, etc.[5, 10].

It has been suggested that when injected as a 40% solution, glucose acts by raising the osmotic pressure and so causing cell dehydration. In this way the intracellular edema of the brain cells [2,3, 9] is overcome. This view has been supported by B. A. Tselibeev [5] who injected isotonic or hypotonic glucose solution. Instead of injecting 20 ml of 40% glucose, he gave 160 ml of 5% or 300 ml of 2.5% solutions, and found that the effect was very much smaller, and often that the coma was not interrupted.

In spite of the general acceptance of the theory of the hyperosmotic action of glucose in insulin coma, no reports have been made on determinations of the osmotic pressure of the blood following the injections.

We have studied osmotic changes in the plasma following intravenous glucose injections during insulin coma in 8 patients suffering from schizophrenia or other psychoses who were undergoing insulin therapy.

METHOD

The injection was given into the vein of one arm, while samples of blood were withdrawn from a vein of the opposite limb; 1-2 ml samples of blood were taken at intervals during the insulin coma, before, during, and immediately after the injection of the 40% glucose solution, and subsequently after 2,5,10-15,and 20-30 min. Osmotic pressure was measured by determination of the freezing point, and the blood glucose by Hagedorn-Jensen's method.

RESULTS

It was found that intravenous injection of 20-40 ml of 40% glucose solution caused no change in the blood osmotic pressure.

Treatment of insulin coma using P. F. Malkin's method [1], by giving a large amount of glucose (80-100 ml of 40% solution), which caused a considerable hyperglycemia, did not change the osmotic pressure (Table 1). Similar results were obtained in 19 other tests.

In order to find by what means the osmotic pressure was maintained constant, the determination of the blood sodium was made in the same samples, by flame photometry. It was found that injection of 20 ml of 40% glucose solution causes no appreciable change in the sodium concentration. Some reduction in blood sodium is caused by injecting 40 ml of 40% glucose during the period of hyperglycemia, and a still greater change results from injecting 80-100 ml of this solution (Table 1).

TABLE 1

Osmotic Pressure and Blood Sodium in Treatment of Insulin Coma by Intravenous Injection of 80 ml 40% Glucose Solution

Time of determination	Osmotic pressure (in milliosmols), normal 300-330	Concentration in blood		Theoretically calculated value (in milliosmols)	
		Glucose (in mg %), normal 80-120	Sodium (in mequiv), normal 130-145	Increase in partial glucose osmotic pressure	Reduction of partial osmotic pressure of sodium
Insulin coma	322	26	142	—	—
During injection	322	556	138	+30	—7
At end of injection	328	385	127	+19	—21
After 2 minutes	322	310	131	+15	—11
After 5 minutes	328	304	123	+15	—34
After 20 minutes	322	96	128	+3	—21
After 30 minutes	328	81	130	+3	—21

TABLE 2

Blood Sodium, and Its Excretion in the Urine and into the Intestine during Insulin Coma Treatment by Injection of 40% Glucose

Time of determination	Concentration in blood		Urine			Amount in intestine			Total Na excreted (in mequiv per min)
	glucose (in mg %)	sodium (in mequiv per liter)	diuresis (in ml/min)	Na concentration (in mequiv/liter)	excretion of Na (in μ equiv/min)	volume excreted (in ml/min)	Na concentration (in mequiv/liter)	Na excreted (in mequiv per min)	
Insulin coma	33	130	0,35	8	3	0,45	84	38	41
During injection	115	125	0,35	9	3	0,35	86	23	26
After injection	416	118	0,25	8	2	0,30	88	26	28
After 4 minutes	211	125	1,00	4	4	0,20	88	17	21
After 8 minutes	138	125	0,80	3	2	0,15	88	13	15
After 12 minutes	129	126	0,50	4	2	0,12	88	11	13
After 20 minutes	107	128	0,20	7	1	0,07	86	6	7

It can be seen from the calculations in Table 1 that the extent of this reduction (or, more accurately, the reduction in the amount of sodium chloride) corresponds quite well to the increased glucose concentration.

It appears that the reduction in the blood sodium is caused by its rapid excretion with the urine or by its passage into the intestine. This was confirmed by an experiment in which fistulas were established on a dog in the bladder and duodenum. Insulin shock was produced, during which 40 ml of 40% glucose solution was injected. The results of the experiment are shown in Table 2.

It can be seen from Table 2 that after the glucose injection there is a reduction in the urinary sodium. The sodium concentration in the intestine remains unchanged, but the amount of fluid obtained through the fistula is reduced. The excretion of sodium into the urine and intestine is therefore not increased, but actually decreased.

TABLE 3

Blood Changes Caused by Injecting 80 ml of 40 % Glucose Solution Intravenously as Insulin Coma Therapy

Time of determination	Osmotic pressure (in milliosmols)	Concentration in blood			Reduction of protein (in % of original value
		glucose (in mg%)	Na (in mequiv per liter)	Protein (in %)	
Insulin coma	338	28	131	7,35	—
During injection	333	205	128	7,35	0
At end of injection . . .	338	292	125	6,51	—11,5
After 2 minutes	333	319	123	6,51	—11,5
After 4 minutes	338	327	125	6,60	—10,2
After 6 minutes	328	350	122	6,71	—8,7
After 20 minutes	322	296	124	6,71	—8,7
After 30 minutes.	338	202	125	6,90	—6,1

TABLE 4

Osmotic Pressure and Blood Sodium as Affected by Injection of 40 ml 10% Sodium Chloride Solution during Insulin Coma

Time of determination	Osmotic pressure (in milliosmols)	Blood sodium (in mequiv per liter)
Insulin coma	328	136
Immediately after injec.	381	175
After 2 minutes.	333	142
After 4 minutes.	328	132

It must be supposed that the osmotic pressure is maintained constant during glucose injections by a redistribution of water between the intra- and extracellular fluids, and that it is on this account that in hyperglycemia, the necessary amount of water flows from the cells into the tissue fluid and thence into the blood. The truth of this conclusion may be tested by determining the refractive index, which is a measure of the total protein content of the plasma. The following results were obtained.

After intravenous injection of glucose, at the period of maximum hyperglycemia , the total protein content in the plasma was noticeably reduced. The results of one of 12 such tests are shown in Table 3.

The observed reduction in blood sodium may be explained as being due to the reduced concentration resulting from the hydremia.

Having shown that the therapeutic action of glucose injection is not due to an increased osmotic pressure we decided to find the effect of an intravenous injection of 40 ml of a 10% sodium chloride solution. In contrast to the published reports, we found that not in one of the 7 subjects to whom the injection was given was there any therapeutic effect, or any noticeable reduction in the depth of the coma. At the same time, immediately after the injection there was a marked increase in the blood osmotic pressure, and an increased blood sodium. But even after 2-3 minutes, both of these quantities returned to their initial levels (Table 4).

Thus, injection of 20-100 ml of 40% glucose solution during insulin coma caused no change in osmotic pressure, despite the marked hyperglycemia. The pressure remained unchanged on account of the dilution of the blood which occurred simultaneously and which was demonstrated by the reduction in plasma protein.

It is probable that during the period of hyperglycemia, the hydremia is caused by passage of water from the cells into the tissue fluid. According to A. S. Troshina [4], dehydration of cells in glucose solutions is not caused by osmosis, but by the action of this substance on the sorptive properties of the protoplasm. From this point of view, the constancy of the osmotic pressure which we have demonstrated during the glucose injection agrees with the idea that the reason for the arrest of the insulin coma is the elimination of the hypoglycemic intracellular edema. The effect of the glucose injection may be due to its special dehydrating action on cells, including brain cells, which it exerts quite independently of its osmotic pressure.

The absence of any effect on injecting hypertonic sodium chloride solution, in spite of the increased osmotic pressure, affords yet further evidence for the truth of this view.

SUMMARY

Intravenous injection of 20-100 ml of a 40% glucose solution during insulin coma causes no change in the blood osmotic pressure, despite the considerable hyperglycemia. The osmotic pressure of the blood is maintained constant on account of a hydremia which develops simultaneously, as indicated by a decreased plasma protein. A short-lasting osmotic pressure rise resulting from the injection of 40 ml of 10% sodium chloride solution does not interrupt insulin coma. The arrest of the coma by glucose may be due to its specific action on the sorptive properties of the cellular colloids, an effect which is independent of osmotic pressure and which counteracts the hypoglycemic intracellular edema of the brain cells.

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THE PATHOGENESIS OF PULMONARY EDEMA INDUCED BY SILVER NITRATE

COMMUNICATION I. THE REASON FOR CIRCULATORY DISTURBANCES IN PULMONARY EDEMA

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Numerous clinical and laboratory observations have shown that pulmonary edema is usually associated with grave circulatory disorders.

In many kinds of toxic pulmonary edema, there is nothing to explain the circulatory disturbances or their relation to the cause of the edema. As an example, we may consider pulmonary edema induced by adrenalin [10,11, 12, 14], ammonium chloride [8, 13], silver nitrate [4,5], and alloxan [7, 9].

In the present investigation, an attempt has been made to find the reason for the circulatory disturbances occurring in pulmonary edema induced by silver nitrate.

METHOD

Adult dogs of both sexes were used. After being fixed to a vivisection table for 30-40 minutes, all the animals received a subcutaneous injection of 0.01 g/kg of morphine hydrochloride. A 0.4% aqueous solution of silver nitrate was freshly prepared for the experiment, and 3.2 mg/kg were injected intravenously.

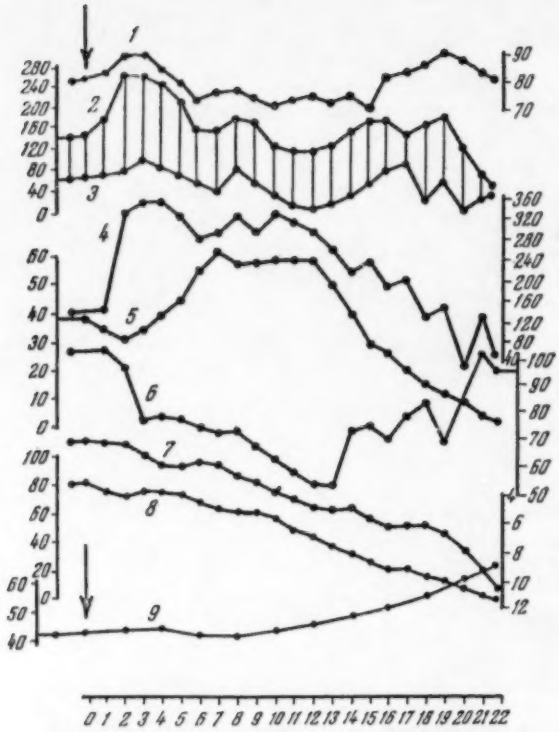
The arterial pressure, the pressure in the right ventricle or pulmonary artery as obtained by a catheter, and the respiration, were recorded on a kymograph. In addition, measurements of the pressure in the inferior vena cava were made (by catheterization) and of the arterial oxygen saturation as determined by a photoelectric oxyhemoglobinometer.

In some experiments, additional recordings of the pressure in the left atrium were made after opening the thorax and introducing a cannula into the auricular appendage. In some of the dogs, erythrocyte volume was measured with a hematocrit.

After the animals had died, a macroscopic and microscopic examination of the viscera was made. Measurements were made of the ratio of the lung weight of the body and heart, as well as of the ratio of the weight of the dry residue to that of the moist pulmonary tissue. In all, 24 experiments were carried out on 24 dogs. To establish normal weight indices, the corresponding measurements were made on 7 control animals, killed by an electric current.

RESULTS

After the injection of silver nitrate, all the animals died within 11-30 minutes, with clear signs of pulmo-



Change of certain hemodynamic and other indices in response to injection of silver nitrate (arrow shows moment of injection). Curves, from above downwards: 1) pressure in inferior vena cava in mm of water; 2-3) pressure in right ventricle, in mm water (systolic and diastolic); 4) pressure in pulmonary artery in mm water; 5) respiration rate, per minute; 6) pressure in left auricle in mm water; 7) pressure in common carotid artery in mm mercury; 8) percentage oxygen saturation of arterial blood, arbitrary units (an increase in the number denotes a reduction in the percentage saturation); 9) erythrocyte volume (hematocrit number).

nary edema. After 1-2 minutes from the beginning of the injection, serious circulatory disturbances occurred, as well as changes in many of the other indices measured.

The figure shows graphically the average values for each recorded quantity. The results of all the experiments are referred to a single time scale which represents the average survival time.

It can be seen from the figure that after the injection, there was a marked increase of pressure in the pulmonary artery. Equally sudden was the fall in pressure in the left auricle. The great reduction in the oxygen saturation of the arterial blood, the increase in respiration rate, and the thickening of the blood (as indicated by the hematocrit number) all of which signs indicate the developments of edema, occurred somewhat later.

Postmortem and microscopical examination showed that in all cases there was a severe pulmonary edema and hyperemia.

Table 1 shows the average values of figures for the weight changes in the lungs.

From the results obtained it can be seen that the pulmonary edema following the intravenous injection of silver nitrate is associated with severe circulatory disorders.

Four basic mechanisms may be concerned in their development: a) the circulatory disturbance may developed as a result of left ventricular failure; b) it may result from the edema (pressure on the pulmonary vessels by the accumulation of exudate); c) silver nitrate alters the physical properties of the blood, and may produce clots within the vessels [2, 5, 6]; possibly therefore, the chief cause of the pulmonary congestion is the formation of emboli in the branches of the pulmonary artery; d) finally, it may be that the circulatory disturbance is due to spasm of the pulmonary vessels. Thus, it is known that solutions of silver nitrate may constrict the vessels of isolated organs [3] and the mesenteric vessels of the frog [1].

We have excluded left ventricular failure as a cause, because of the low pressure in the left auricle. A reduced output from the left ventricle with increased work by the right (see figure) is scarcely possible without a raised pressure in the left auricle. In our experiments, on the contrary, after injecting the silver nitrate solution the pressure in the left auricle was reduced.

Circulatory disturbances could not be brought about by the edema which develops, because they occur too rapidly after the injection. In most experiments, even one minute after the beginning of the injection, there has been a considerable increase in pressure in the pulmonary artery (see figure), and sometimes the effect occurs after only 12-15 seconds. It is hard to imagine that by this time the edema could have develope[illegible]fficiently for the accumulated exudate to cause any appreciable pressure on the pulmonary vessels.

Secondly, at the time of the maximum pressure increase in the pulmonary artery, th[illegible]f

TABLE

Ratio of Lung Weight to Body and Heart Weight, and Ratio of Dried Lung Residue to Raw Lung Weight

Animal	Number of animals	Ratio of lung weight, in g to body weight, in kg (M ± m)	Ratio of lung weight to heart weight, in g (M ± m)	Dried lung residue as % of weight of raw tissue (M ± m)
Experiment	20*	38,55±1,60	4,68±0,25	13,40±0,35
Control	7	9,35±0,83	0,96±0,05	20,10±20,41

* Four experiments in which artificial respiration was used are not included.

respiration, oxygen saturation of arterial blood, and hematocrit number, all of which indicate the extent of the edema, have not yet shown any appreciable change (see figure).

A. M. Kotovshikov [5] considered the possibility of the pressure in the pulmonary artery being raised after silver nitrate injections, through the action of a large number of embolisms in the pulmonary circulation containing dense masses of changed blood. He concluded that the possibility of embolism formation depends on the amount of silver nitrate injected. With comparatively large doses, clots of coagulated blood could be observed under the microscope, and he thought that these could give rise to embolisms. He found no such clots with small doses.

The amounts of silver nitrate which we used for producing pulmonary edema corresponded approximately to those which did not cause blood changes in A. M. Kotovshikov's experiment, and were considerably smaller than those which caused blood changes which could be observed microscopically. Also, after the intravenous injection, microscopical examination revealed no trace of metabolisms.

From what has been described, it may be supposed that embolisms of the pulmonary circulation are not the cause of the profound circulatory disturbances observed. However, these could be fully accounted for by spasm of the pulmonary vessels.

In our opinion, spasm of the pulmonary vessels is the principal cause of the circulatory disturbance resulting from intravenous injection of silver nitrate in dogs.

SUMMARY

Experiments were performed on dogs in which silver nitrate solution was injected intravenously to induce acute pulmonary edema. Recordings were made of arterial and venous pressure, pressure in the left auricle and pulmonary artery, respiration, arterial blood oxygen saturation, and hematocrit ratio. Postmortem determinations were made of lung weight, ratio of body to heart weight, and ratio of dried to raw lung tissue.

The pulmonary edema which developed was accompanied by acute circulatory disturbances; these were not the direct effect of the edema, nor did they result from the denaturing effect of silver nitrate on the blood, nor from left ventricular failure. It was thought that the circulatory disorders resulted from spasm of the pulmonary vessels.

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HEMOPOIETIC REACTION TO THE INJECTION OF CAMPOLON UNDER DIFFERENT PHYSIOLOGICAL CONDITIONS

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Many workers have shown [2, 3, 5] that the nervous system exerts a continuous and active control over the details of processes occurring in the hemopoietic system.

In the present study we set out to determine the effect on the hemopoietic system, under different physiological conditions, of a certain preparation known to have an effect on it.

The substance chosen was campolon, which is widely used in clinical medicine. It was chosen because several authors had demonstrated the direct utilization of hemopoietic substances by the reticular tissue of the bone marrow [4]. We were also influenced by results which we had obtained earlier [1], concerning the part played by the nervous system in mediating the effect of campolon on hemopoiesis. The response of the hemopoietic tissue to compolon injections was studied under normal physiological conditions, and after severe hemorrhage.

METHOD

Two sets of observations were made. In the first, the experiments were carried out on 3 male dogs. An injection of 0.2 ml per kg of compolon was given. The effects were noted by studying the condition of peripheral blood before the injection, and 1, 2, 3, 4, 5, and 6 hours after it. Blood samples were taken from a small cut in the ear. Erythrocyte, leukocyte, and differential white cell counts were made. Severe anemia was induced by bleeding 300–400 ml of blood from a vein.

The second set of observations was made on human subjects clinically. There were 12 patients with acute anemia following childbirth, abortion, etc., and they had been treated by a single injection of 3–4 ml of compolon. Their hemoglobin varied from 40–60 %, and the erythrocyte count from 2.5 to 3.5 million. As a control, simultaneous observations were made on 6 patients with normal blood (hemoglobin from 75% upwards, erythrocyte count higher than 4 million). They were also given the same dose of campolon. Blood samples were taken before giving the injection, and 3 and 5 hours afterwards.

RESULTS

Campolon injection almost always increased the production of blood cells in the experimental animals. An increase in the number of leukocytes in the peripheral blood occurred after approximately 3 hours. There was a further increase subsequently. In some experiments, 5 hours after the injection, the leukocyte count had more than doubled (Fig. 1). The differential white cell count showed an increased number of neutrophils,

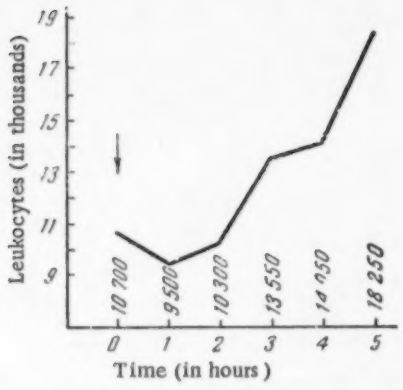

Fig. 1. Change in the number of leukocytes following an injection of campolon (↓) into the dog Orel, under normal conditions.

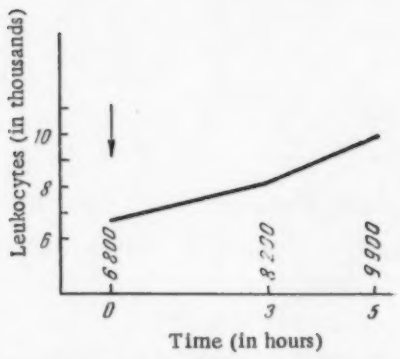

Fig. 2. Change in the number of leukocytes after giving an injection of campolon (↓) to the patient S. V., having a normal blood count.

with a shift to the left. A single injection caused no reproducible changes in the number of red cells.

Similar results were obtained in patients with normal blood. A single campolon injection caused a marked increase in the number of leukocytes. The increase occurred 3 hours after the injection, and after 5 hours, the number still continued to increase (Fig. 2). The differential white cell count showed some increase in the number of neutrophils, with a shift to the left. It must be noted that the change in the number of white cells was not so well shown in the patients as in the animals. This effect is no doubt due to the smaller amount of campolon given to the human subjects.

Having determined the effect of campolon on the blood under normal conditions, we then proceeded to find the effect produced in anemia.

The animals were bled as follows. In the dog Sokol, before bleeding, the blood count showed 5,680,000 erythrocytes and 13,300 leukocytes per ml, with a hemoglobin of 82. On the day after withdrawing 350 ml of blood, the corresponding figures were 4,085,000 erythrocytes, 20,500 leukocytes, and a 61% hemoglobin. In the dog Orel, the normal blood count was 4,870,000 erythrocytes, 12,400 leukocytes, with a hemoglobin of 76%, and on the day after bleeding these figures had changed to 3,400,000 erythrocytes, 14,500 leukocytes, and a hemoglobin of 54%.

Giving campolon after bleeding produced a characteristic reaction from the bone marrow, and after 2 or 3 hours, the number of leukocytes had been reduced. The leukopenia was long lasting, and continued to increase in the following hours. It was found that the reduction in the number of leukocytes was well shown for the first few days after the bleeding. Later, as the blood count returned to normal, the reaction of the bone marrow to campolon injections gradually returned to normal. Fig. 3 shows the change in the number of leukocytes in the experiment in which campolon was injected.

Observations on patients with acute anemia gave similar, but less well shown results, as shown in Fig. 4.

In this way, it was shown that the response of the hemopoietic system to campolon injections depends on the physiological condition of the animal. Normally, it causes an activation, but in acute anemia it inhibits hemopoiesis.

We suggest the following interpretation of the results. Normally, when the bone marrow functions in accordance with the level of vital activity of the organism, a specific stimulant such as campolon will increase its activity.

After severe blood loss, hemopoiesis is increased, because the bone marrow is stimulated by erythrocytic and metabolic and breakdown products. Consequently, the hemopoietic system is maximally activated. The

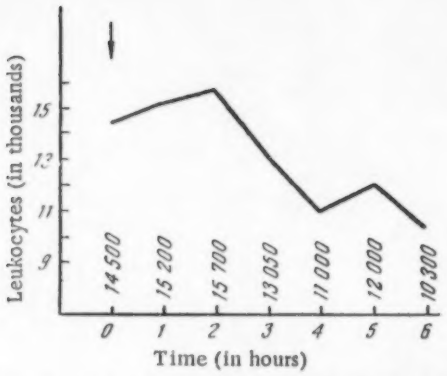

Fig. 3. Change in the number of leukocytes on giving the dog Orel a campolon injection (↓) following large blood loss.

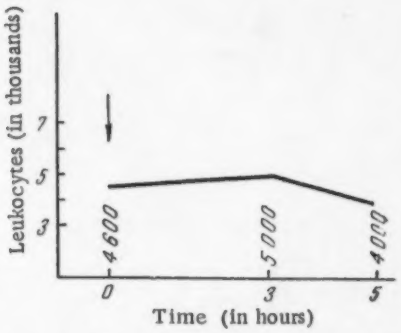

Fig. 4. Change in the number of leukocytes on giving the patient G. F., with acute anemia, an injection of campolon (↓).

superimposition of the strong chemical stimulus exerted by campolon, which stimulates hemopoiesis, brings about unfavorable conditions, with the result that hemopoiesis is actually reduced.

SUMMARY

A study was made of the specific reaction of the hemopoietic system to the injection of campolon in normal animals and in those which had suffered severe blood loss. Two sets of experiments on animals were carried out, and clinical studies of human patients were made. In almost every case, campolon injection induced a hemopoietic response. The number of leukocytes in the peripheral blood began to rise about 3 hours after the injection. There was an increase in the number of neutrophils, with a shift to the left. When there had already been a severe blood loss, campolon caused a definite leukopenia, which was especially evident in cases with acute anemia. The results show that there are two possible reactions of the hemopoietic system to stimuli of different intensities.

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CARDIAC ACTIVITY CHANGES IN TYPHOID FEVER IN DOGS

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Experimental analysis of the physiological mechanisms controlling the course of dysentery, staphylococcal infections, and diphtheria in dogs of different ages has revealed a cyclical reaction, whose features are correlated with age. In the previously mentioned infections, the cyclical reaction displays characteristic changes in the different phases, which are particularly well shown in the reaction of the nervous centers controlling the cardiovascular system [3, 4, 14].

In the present investigation, we studied the action of the typhoid toxin with a view to understanding the characteristic symptoms and changes in cardiac activity in human typhoid patients of different ages.

METHOD

The experiments were carried out on 38 adult dogs, 12 puppies less than 22 days old, and on 4 ages 3-4½ months. Typhoid was induced by using a two-phase antigen prepared in the N. F. Gamaleya Institute of Epidemiology and Microbiology. This consisted of a concentrate of a whole culture (liquid portion and dead bacteria).

In a previous communication we have shown that typhoid toxin produces precisely the same intestinal symptoms in adult dogs as are found in adult human subjects [15]. Two antigens were available, prepared from two different typhoid strains. Antigen 1 was the most toxic, the lethal dose being about 10 mg per kg body weight, while for antigen 2, it was 15-20 mg per kg. The antigens, diluted in saline, were injected subcutaneously or intravenously. Careful observations on the condition of the animals were made. Temperature and respiration rates were recorded. Cardiac changes were recorded from lead 2 of an EKP-4 electrocardiograph. The ECG was first taken several hours before the injection, and subsequently at frequent intervals during the whole course of the illness, until recovery or death, according to the dose of toxin given. The animals were not anesthetized or restrained. Four of them were given a preliminary injection of 10 mg/kg of morphine.

RESULTS

From the results obtained, the animals may be divided into two groups. The first consists of puppies less than 22 days old, and the second of adult animals of 3 months or above. We will first describe the results for the older age group. In all of them, both antigens (1 and 2) produced the same quantitative reaction. The only difference was in the size of the dose required to produce a particular reaction. Antigen 1 was the stronger of the two (see 'Method'). The typhoid toxin was injected after the normal resting heart rate, which varied from 70 to 90-100 beats per minute, had been established. In the resting condition, respiratory arrhythmia might be present in greater or lesser degree.

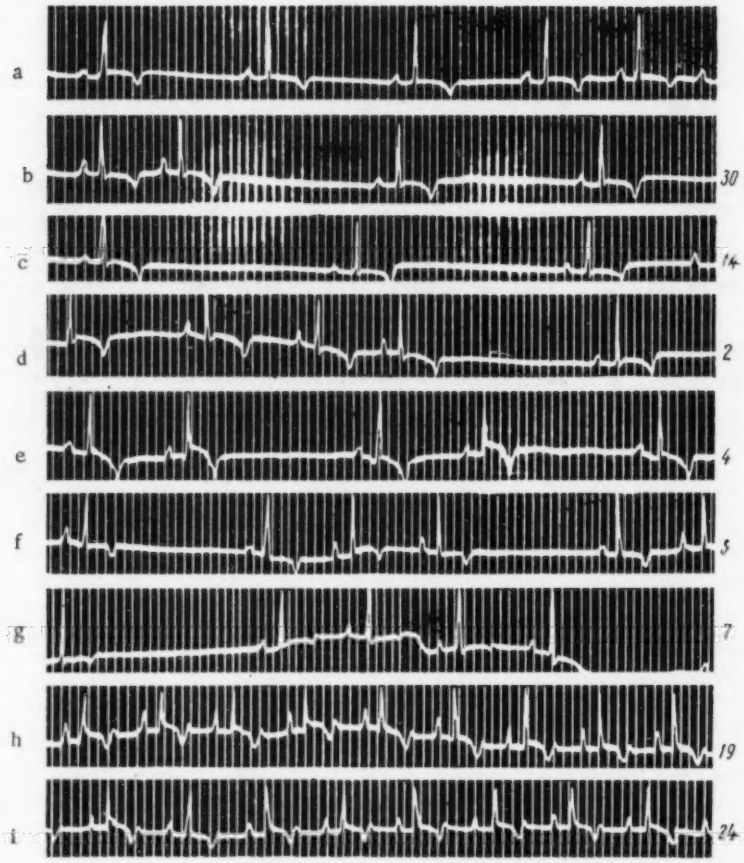

Fig. 1. Electrocardiogram of an adult dog infected with typhoid. a) Before the injection of typhoid toxin; b, c, d, e, f, g) first phase of the reaction; h, i) second phase.

If a sublethal dose of 4-6 mg of Antigen 1, or 6-10 mg of Antigen 2 was now injected, after 10-20 minutes there was a marked slowing of the heart rate, and marked respiratory arrhythmia. The rate fell to 60 or even to 50 beats per minute. Respiration was also slowed. In addition, there was a marked reduction in the amplitude of the P and T waves, and, simultaneously, some increase in the R wave. In the resting condition, the T wave was given negative, and after the injection, it remained negative and increased in amplitude. This first phase of the reaction lasted from 6 to 12 hours, and in some cases as long as 20 hours. The greater the dose of toxin injected, the shorter the duration of the first phase; however, in all cases, this first phase lasted longer than has been reported in staphylococcal infections, dysentery, and diphtheria.

The second phase follows immediately after the first, when there is an increase in heart and respiration rate. The heartbeat rises to 120-150 per minute. Respiratory arrhythmia ceases. The amplitudes of the P and T waves increase, while the R wave is reduced. This phase may last any time from a few hours to several days; the greater the dose, the greater the duration (Fig. 1).

When phase 2 has been completed, there is once more a marked reduction in heart rate to a value 15-30 beats per minute below the original resting rate. The respiration rate is also greatly reduced. A marked respiratory arrhythmia also returns. The P and T waves become reduced, while the R wave is greatly increased. This phase corresponds to the condition described by A. A. Koltypin [10], who referred to it as the vagus phase in the acute stage of several infectious diseases in children. The occurrence of this phase indicates the cyclical nature of the reaction to typhoid toxin. Subsequently, there is a gradual return to the initial resting condition.

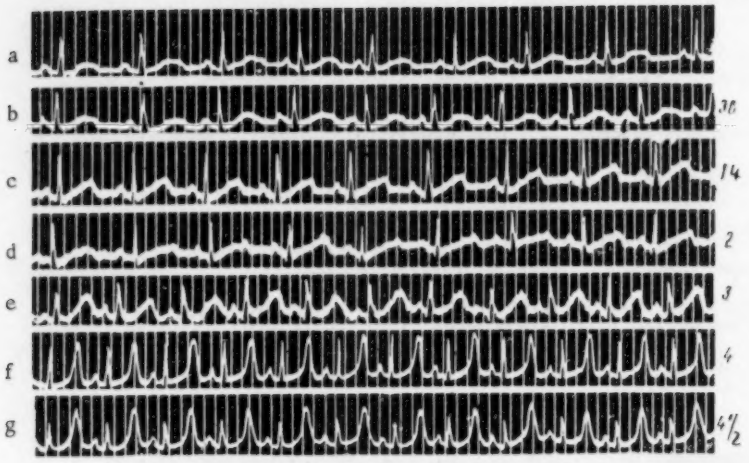

Fig. 2. Electrocardiogram of 22-day-old puppy injected with typhoid toxin. a) Before injection; b, c, d, e) initial stage of first phase of reaction; f, g) phase 1 maximally developed.

The duration of the whole cycle varies from 2 to 4 days. When small doses of 2-3 mg/kg of Antigen 1 are given, the reaction does not extend beyond phase 1.

We must now consider the cause of the change in the heart rate and in the amplitudes of the ECG waves. Information on this point was obtained by giving an intravenous injections of 0.1 mg/kg of atropine to 9 dogs, 30 minutes before the injection of the toxin; this dose of atropine was sufficient to block tonic cardiac vagal impulses. During the course of the infections of small amounts of atropine were given at intervals of $2\frac{1}{2}$ - 3 hours. In the atropine treated dogs, the phasic changes in heart rate and ECG waves were completely absent. During the whole period of observation, the heart rate remained constant.

Thus, the natural conclusion is that just as has been shown for staphylococcal, dysenteric, and diphtheretic infections, in both the first phase and in the phase when the changes in heart rhythm and ECG waves recur, the tonic action of the vagal cardiac center is in a condition of increased activity (first optimum reaction of this center).

In the second phase of the reaction, in which the heart rate is increased, there is an inhibition of vagal cardiac innervation (first pessimum of the reaction of this center). The amount by which the heart rate is increased in the second phase depends on the degree to which the vagal cardiac center is inhibited. When lethal doses are injected, the first phase is considerably shortened. Also, the second phase shows a more marked heart rate increase, up to 160-200 per minute.

The third phase follows immediately after the second. Unlike the phase 3 which has been described, it consists of a short cardiac spasm, or syncope, lasting from 3 to 50 seconds. Finally, the heart again begins to beat at the very slow atrioventricular rhythm (vagus escape). The R wave is enterely missing from the ECG. The whole of this phase, comprising syncope and the subsequent transition to the atrioventricular rhythm, does not last more than 3-7 minutes. At this period, breathing usually stops for a period before the last breath is taken.

The third phase is also vagal in origin, and is due to a marked increase in the tone of the vagal cardiac center; it does not occur in atropinized dogs. By analogy with the corresponding phase in other lethal bacterial infections, the third phase may be considered as the second optimum of the reaction of the vagal cardiac center. At the end of the third phase, the heart goes over to the sinoauricular rhythm, which does not change after section of the vagi (second pessimum of the reaction of the vagal cardiac center). In some experiments, in the third phase, fibrillation of the heart occurred.

In puppies of the younger age group, subletal doses of typhoid toxin do not cause a slowing of the heart rhythm, as in the older animals, but an immediate increase of 30-60 beats per minute. The increased rate is

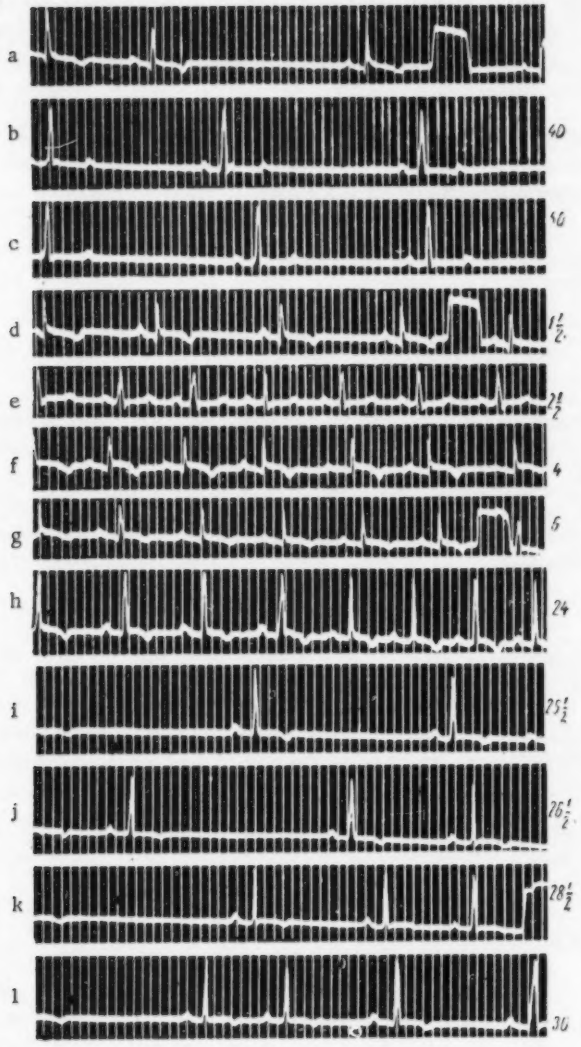

Fig. 3. Electrocardiogram of an adult dog injected with dysenteric toxin. Reactions; e, f, g) second phase; h) beginning of phase; a) before injection of dysenteric toxin; b, c, d) first phase of recovery; h, i, j, k) recovery phase.

maintained for the whole period of the toxin action, and may last from 8 to 30 hours according to the dose of toxin given; subsequently, there is a gradual return to the initial condition. Fig. 2 shows a series of ECG recordings taken from a 22-day-old puppy, before and after injecting typhoid toxin.

Thus, in young puppies given a sublethal dose of toxin, the whole of the reaction is confined to the first phase. This result is readily understood when it is appreciated that in young puppies, the heart rate is not controlled from the vagal center, but from the center for cardiac sympathetic innervation [1, 6]. The increase heart rate caused by the injection of typhoid toxin into young puppies must be attributed to a stimulation of the cardiac sympathetic center.

If young puppies are given a lethal dose of typhoid toxin, then immediately after the phase of raised heart rate, there is a progressive slowing of the cardiac rhythm, and this represents one of the components of protracted

collapse, as frequently described by I. A. Arshavskii and his co-workers in many cases of bacterial and chemical poisoning. In adult dogs, in the raised heart rate induced by large amounts of typhoid toxin, there is an inhibition of the vagal cardiac center, whereas in young puppies, the slowing of the heart rate caused by large doses is due to inhibition of the sympathetic center.

The results obtained in the experiments on young puppies explain why the bradycardia which is so typical of typhoid in adults [5-9] fails to develop in young children, especially in those of preschool age. We must ask, then, whether the typical adult bradycardia represents a specific effect of typhoid toxin acting as a stimulus. Were this to be the case, then evidently bradycardia would occur in the young also. The particular form the reaction takes depends not only on the nature of the stimulus, but also on the age of the human or animal infected.

It was shown above that bradycardia in adult dogs is the primary response not only to the action of bacterial toxins, but also to that of many chemical substances. We require to know what features are specifically induced by the bacterial toxin.

Injections of His-Flexner dysenteric toxin were given subcutaneously to 6 adult dogs; 3 received a sublethal, and 3 a lethal dose. With the sublethal dose, the typical phasic reaction occurred, there was a bradycardia, tachycardia, and then a return of the bradycardia, and the corresponding changes in the ECG waves occurred at each phase, just as we have already described for typhoid infection (Fig. 3). When lethal doses were given, the same sequence of four phases of the reaction of the vagal center, as has already been described for lethal doses of typhoid toxin, was observed.

The results of this set of experiments are in line with the changes in cardiac activity observed to occur in dysentery [4, 14].

What are the distinctive features of the reaction of the heart to typhoid and dysenteric toxins ? From his own results, and those of his co-workers, I. A. Arshavskii [2] concluded that they consist essentially of a variation pf the nonspecific normal parabiotic reaction. It was shown above that when sublethal doses of typhoid toxin are given, the first phase of the bradycardia lasts for from 6 to 20 hours. With sublethal doses of dysenteric toxin, the duration of the first bradycardial reaction varies from 30 minutes to 1½ hours.

The specific feature of the typhoid toxin on adult animals is the unusual length of the initial bradycardia.

SUMMARY

Characteristic changes in cardiac activity due to the action of typhoid toxin in full-grown dogs and young puppies are described. The response of adult dogs to the intravenous or subcutaneous injection of sublethal doses of typhoid toxin takes the form of a cyclic reaction involving the following phases: first bradycardia, second tachycardia, and third a restoration of the original bradycardia which terminates in a return to the initial condition. All these phases reflect the changes in the intensity of the tonic excitation of the vagus center of cardiac innervation. In previously atropinized dogs the above phases of the reaction are absent. In young puppies the cyclic reaction to intravenous or subcutaneous injections of the toxin is limited to the tachycardia phase only, which reflects the rise in the intensity of the tonic excitation occurring in the center of the sympathetic cardiac innervation. The action of the typhoid toxin in lethal doses not only alters the correlated duration of the reaction phases cited, but also gives rise to additional ones, both in full-grown dogs and in young puppies.

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ACTION OF HISTAMINE ON THE CAROTID SINUS OF THE DOG

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Histamine plays a definite part in the development of various kinds of shock. When injected parenterally, it produces a condition which in many respects resembles anaphylactic shock.

An important feature of its action is its influence on effectors but scarcely any attention has been paid to its possible influence on reflex effects from the interoceptors; nevertheless, Lewis [8], as far back as 1927, showed the importance of the axon reflex in the action of histamine on capillaries. According to Landgren and others [7], 2-50 γ of histamine produces no stimulant effect on the chemoceptors of the carotid sinus. However, the same amount of histamine is effective in stimulating the receptors of the intestine and spleen [3]. A greater amount (5 mg) causes marked reflex changes in respiration and arterial pressure through acting on the interoceptors of the limb vessels [10].

Published reports [2, 4, 5] have shown that substances which affect the contraction of the smooth muscle of blood vessels, also exert an influence on the mechanoreceptors of the carotid sinus and aorta.

These receptors are intimately concerned in the maintenance of the arterial pressure [3], and because histamine can influence the contraction of the smooth muscle of many organs, we decided to examine its action on the depressor receptors of the carotid sinus.

METHOD

The influence of histamine on arterial pressure was studied in 15 dogs, 6 of which were sensitized to horse serum 16-23 days previously; they received 0.2 ml of serum per kg subcutaneously on two successive days.

Morphine-pentothal anesthesia was used. The carotid sinus was exposed, and the arterial pressure measured with a mercury manometer either from the common carotid artery of the same side, or from the femoral artery.

The sinocarotid pressor reflex was induced by lowering the pressure in the carotid sinus by compressing the common carotid. The depressor sinocarotid reflex was produced by a stepwise increase of 20 mm at a time of the pressure of the pressure in the sinus, using a modification of the method of Lim and Chang [9]. The method allowed the increase in volume of the carotid sinus in response to pressure changes to be determined (Fig. 1). The method was accurate to ± 0.0025 ml.

To determine the action on the smooth muscle of the carotid sinus of whole horse serum and of 0.25 ml of 0.1% solutions of histamine and adrenalin, the wall of the carotid sinus and adjacent parts of the carotid artery were infiltrated with these substances.

RESULTS

There was no difference in the reflex response to histamine between the normal and the sensitized animals, and the results for the two groups have been placed together.

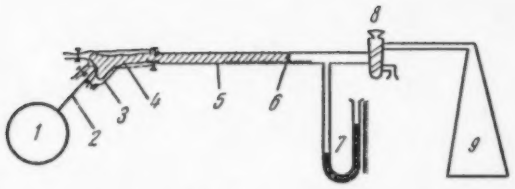

Fig. 1. Diagram of the apparatus for recording the effect of pressure changes on carotid sinus volume. 1) Vasomotor center; 2) nerve supplying sinus; 3) carotid sinus; 4) rubber balloon introduced into carotid sinus; 5) cannula with scale divisions, filled with physiological saline and connected to the balloon within the carotid sinus; 6) meniscus of physiological saline, movement of which indicates variation in sinus volume; 7) manometer; 8) two-way tap connecting cannula either with atmospheric pressure or with compressed air supply; 9) cylinder with compressed air.

In most cases, the effect of histamine on the carotid sinus was to reduce the arterial pressure. In experiments where circulation through the sinus was maintained, there was the possibility of absorption of histamine into the blood stream. However, even when no blood passed through the sinus, infiltrating the wall with histamine also produced a reflex hypotension, (Fig. 3, a).

Section of the sinocarotid nerve or infiltrating the wall of the sinus with novocain solution eliminates the reflex hypotension. The amount by which the pressure fell varied from 4 to 30 mm, representing 3 to 18% of the original arterial pressure. The change lasted from 7 to 20 minutes or more. In one case, there was an increase in arterial pressure of 9 mm which lasted for 1.5 minutes. There were other cases in which no change occurred.

A study of variation of the elasticity of the carotid sinus wall showed that in 60% of the cases histamine produced no change. In the remaining 40% of the experiments, after histamine action it was found that there was a slower increase in volume over the pressure range of 80 to 120 mm, but with higher pressures, the volume of the histamine-treated preparations increased more rapidly (Fig. 2, A). This can be very well seen on the trace for the "volume elasticity" given by Landgren [6] and (Fig. 2, B).

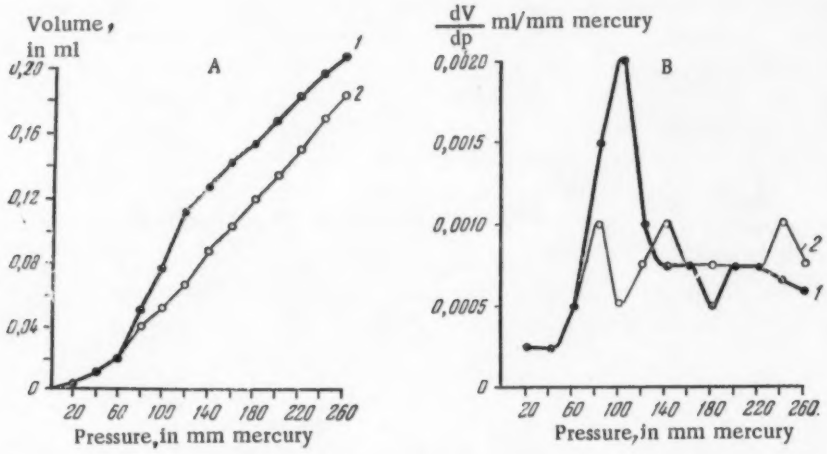

Fig. 2. Effect of histamine on the elasticity of the carotid sinus wall. A) Increase in carotid sinus volume due to increased internal pressure; B) change in the "volume elasticity". 1) Before, 2) after the action of histamine.

Although in histamine hypotension the pressor response to a lowered carotid sinus pressure is reduced, we never found it to be enterely absent, as did A. D. Ado and M. A. Erzin [1] after the anaphylactic reaction, and Heymans [5] after the action of adrenalin on the carotid sinus.

In comparing the reflex hypotensive effects of histamine and adrenalin, it is immediately apparent that adrenalin is the more active substance. It produces a greater change which lasts for a longer time. When histamine is added to a carotid sinus already infiltrated with adrenalin, no blood pressure change occurs.

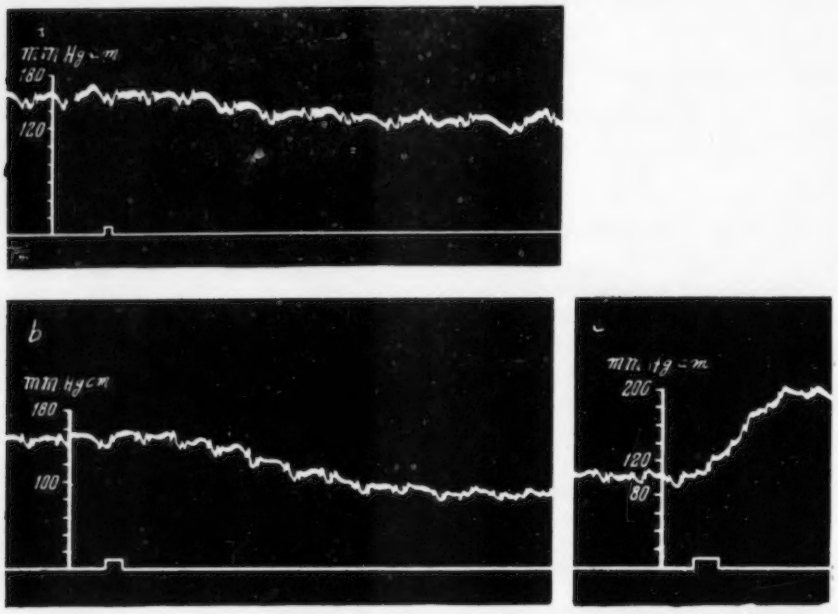

Fig. 3. Arterial pressure change on infiltrating the wall of the isolated carotid sinus with histamine and with adrenalin. Dog, weight 15 kg. Vagi sectioned in neck. Tracing on millimeter paper, speed 1 mm/second. a)Infiltrating the wall of the isolated carotid sinus with 0.25 ml of 1: 1000 histamine. Reduction of arterial pressure by 30 mm mercury. b) 30 minutes later. Arterial pressure has returned to the initial value. Infiltration of the carotid sinus wall with 0.25 ml of 1 : 1000 adrenalin causes a reduction in arterial pressure of 60 mm mercury. c) 40 minutes after the action of adrenalin. The arterial pressure has not returned to the initial value. Infiltrating the wall of the carotid sinus with 2.5 ml of 0.5% novocain inhibits the reflex hypotension.

On the other hand, a definite hypotensive reflex occurs if adrenalin is added to a carotid sinus already treated with histamine. Similarly, histamine is without effect in sensitized dogs in whom reflex hypotension has been induced by treating the carotid sinus with horse serum.

Thus, histamine is a less effective stimulus for the depressor mechanoceptors of the carotid sinus than is adrenalin or the permissible dose of serum. It seems probable that histamine does not play any important part in the development of the reflex "sinus shock" [1].

SUMMARY

In most instances infiltration of the carotid sinus wall with histamine causes a reflex drop of arterial pressure the intensity and duration of which is lower than in the case of hypotension produced by the adrenalin action on the smooth muscles of the carotid sinus.

No connection was revealed between the carotid sinus wall expansion and the depressor reflex.

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THE ACTIN CONTENT OF THE MYOMETRIUM

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The smooth muscle of the uterus, like other forms of smooth muscle, is known to differ considerably in its protein composition from skeletal muscle. The most characteristic feature of smooth tonic muscle is its low content of actomyosin [2, 3, 4, 5, 7, 9] and its relatively high content of water-soluble myofibrillary proteins.

The study of the fractional composition of the proteins of the myometrium has attracted, in recent years, the attention of many research workers [1, 7, 8, 11 and others]. These authors have confirmed the view that the muscle of the nonpregnant uterus has a low content of contractile proteins — myosin and actomyosin. The presence of considerable quantities of tropomyosin in the myometrium has been pointed out on several occasions [12, 14 and others].

The work of Snellman and Tenow differs from that of other workers in that these authors found considerable amounts of free actin (up to 35-40%) besides a protein complex (which they called actotropomyosin) in extracts obtained from the muscle of the uterus with solutions of high ionic strength. According to these workers' findings, action readily passes into solution even during brief extraction of minced muscle with 0.5M KCl. This view, however, is not in accordance with certain facts.

In the present paper we describe findings from which it will be seen that the proteins extracted from Weber's solution from the smooth muscle of the uterus (both pregnant and nonpregnant) do not have the power to react with myosin preparations to form actomyosin: the viscosity and the dissociation power of extracts of smooth muscle of the uterus during reaction with ATP were not appreciably increased by the addition of myosin solutions to them. This makes Snellman and Tenow's view of the presence of any significant amount of free actin in 0.5 M KCl extracts of the smooth muscle of the uterus most unlikely.

In our experiments, in order to define the protein composition of the myometrium, proteins extracted from the uterine muscle with Weber's solution were subjected to fractionation by means of paper electrophoresis. These investigations also gave no grounds for relating the special feactures of the proteins of the myometrium to the high content of "readily extractable" actin in the smooth muscle of the uterus.

EXPERIMENTAL METHOD

The contractile proteins were isolated from the striped muscle and uterine muscle of rabbits (nonpregnant and pregnant). Myosin was obtained by extraction of minced skeletal muscles for 7-8 minutes with three times their volume of Weber's solution in the cold. Actin was prepared by Straub's method. The actin was activated with the salts KCl and $MgCl_2$ (final concentration of KCl — 0.6M, $MgCl_2$ — 0.0005 M). Saline extracts were prepared from the uterine muscle, carefully freed from mucous membrane. Minced muscle tissue was extracted immediately (or after one preliminary washing with 0.3 M KCl solution) with three times its own volume of

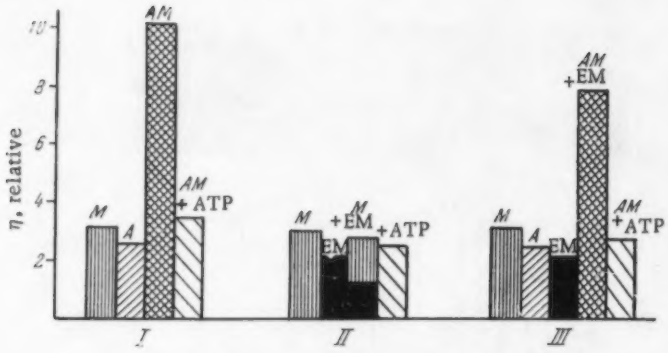

Fig. 1. Changes in the relative viscosity of myosin solution after reaction with actin and with uterine muscle extract. M) Myosin; A) actin; AM) actomyosin; EM) uterine muscle extract.

Weber's solution for 20-22 hours. Myosin and actin solutions from the skeletal muscle, and also saline extracts of uterine muscle, were prepared with closely similar viscosity. The viscosity was measured in an Ostwald viscosimeter at a temperature of +17°C. A 1% solution of ATP was added directly into the viscosimeter. The myosin and actin solutions were added together in proportions of 3:1 by volume (in order to obtain an "artificial" actomyosin), and the uterine muscle extracts and myosin solution were combined in proportions of 1:1.

Electrophoresis of the proteins of the myometrium was carried out under the following conditions. The uterine muscle was cut up finely with scissors, pounded with three times its volume of Weber's solution and extracted in the cold for 24 hours. The suspension was squeezed through 3 layers of gauze and then centrifuged at 4000 rpm. The centrifugate obtained was decanted from the precipitate and dialyzed against a buffer solution for 24 hours. After dialysis, the extract was again centrifuged to ensure complete removal of suspended particles. The protein concentration of the solution was usually 1.2-1.8%. Electrophoresis was carried out in a type LKB 3276 apparatus. The protein solutions, in volumes of 0.03-0.04 ml, were applied to strips of paper measuring 40x 410 mm (Schleicher and Schüll No. 2043 B electrophoresis paper). Electrophoresis was performed in a phosphate buffer at pH = 7.8 and at an ionic strength of 0.35 for 24 hours, with a potential gradient of 4.0-4.5 v/cm and a current of 0.8 ma/cm^2 cross section of paper. The dry electrophoregram was fixed at a temperature of 105° for 15 minutes and then stained in the usual manner with thymol blue. The staining density of the bands corresponding to the different protein fractions was determined by means of a densitometer.

EXPERIMENTAL RESULTS

The results of one of the experiments are shown graphically. As may be seen from Fig. 1(variant 1 of the experiment), myosin solution from skeletal muscles, when mixed with actin solution (in proportions of 3:1) underwent a sharp change in its relative viscosity η as a result of the formation of artificial actomyosin. On the addition of ATP the viscosity of the formed actomyosin fell to the viscosity of the original solutions.

If the myosin solution was mixed with a saline extract of the uterine muscle of a nonpregnant rabbit (according to Snellman and Tenow's findings, this extract contained 35-40% of free actin), in this case no change was observed in the viscosity, even when the myosin solution and the saline extract of the uterine muscle were mixed in proportions of 1:1 (variant II of the experiment). The viscosity assumed an average value, which was determined by the initial viscosity and by the volumes of the solutions mixed. In precisely the same way the viscosity of the solution obtained showed practically no fall after addition of ATP. The insignificant fall in the viscosity which might have been observed after addition of ATP to the solution was due to the presence of a small quantity of actomyosin in the extract of the uterine muscle.

We obtained similar results in experiments with extracts of the uterus of pregnant rabbits.

The supposition that the extracts of the myometrium contained both actin and a hypothetical inhibitor, in the presence of which the reaction of combination of actin with myosin was retarded, was tested by variant III

a.

b

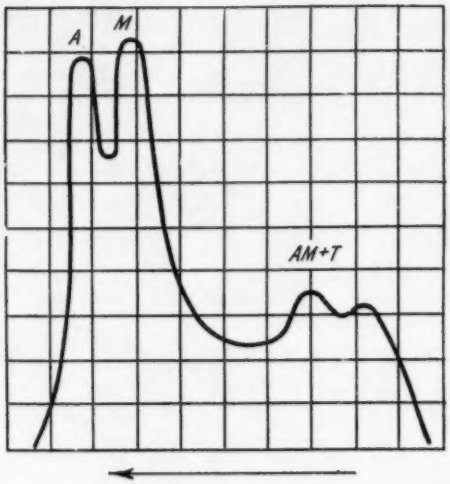

Fig. 2. Separation of the protein fractions of an extract of the uterine muscle of a nonpregnant rabbit (on a strip of paper). a) Stained electrophoregram; b) curve obtained by densitometry of the paper electrophoregram: A) Myoalbumin; M) myogenic fraction ; AM + T) actomyosin + water-soluble myofibrillary proteins.

of the experiment. In this series of experiment myosin solution, actin solution and extract of myometrium were mixed in proportions of 2:1:2. As might have been expected, a highly viscous solution of actomyosin was obtained, which had its relative viscosity sharply reduced by the addition of ATP.

This suggested that Snellman and Tenow's conclusion [12], of the high content of "readily extracted" actin in the myometrium, is incorrect. The distinctive features of the protein composition of the smooth muscle of the uterus were, evidently, in no way connected with the presence of a large amount of actin in muscle of this type.

The results of the electrophoretic investigation of the proteins of the myometrium (Fig. 2), extracted from the uterine muscle by salt solutions of high ionic strength, showed that the proteins of the smooth muscle of the uterus mainly consist of 3 fractions: myoalbumin A, heterogeneous myogen fraction M and actomyosin fraction AM. The last, according to the findings of I. I. Ivanov and his co-workers, consists of a small quantity of actomyosin combined with the water-soluble myofibrillary proteins of the myometrium (fraction T), mainly with tropomyosin, and with a new, water-soluble myofibrillary protein (Δ-protein, X-protein), whose properties have recently been studied by many authors [6, 10, 13, 15 and others]. The electrophoretic mobility of these proteins is close to the mobility of the proteins of the actomyosin complex.

During paper electrophoresis the fraction of water-soluble myofibrillary proteins (fraction T in our terminology) remained on the paper strip at the starting point or in its immediate proximity. During free electrophoresis the order of arrangement of the individual peaks on the electrophoregram of the uterine muscle proteins was changed.

SUMMARY

Proteins extracted by Weber's solution from the smooth muscle of the uterus do not possess the ability to interact with myosin preparations with the formation of actomyosin. This makes highly improbable the assumption of Snellman and Tenow on the presence of any appreciable amounts of free actin in 0.5 M KCL extracts of myometrium.

Investigations conducted by the method of paper electrophoresis demonstrate the presence of 3 fractions in the myometrium extracts with high ionic potential, viz., myoalbumin, heterogeneous myogen and actomyosin. The latter consists of a small quantity of actomyosin (undoubtedly bound with nucleoproteins) and water-soluble myofibrillar proteins (mainly tropomyosin and possibly Δ-protein). Likewise these investigations give no grounds for associating the peculiarity of the myometrial proteins with the high concentration of the "easily extractable" actin in the muscles of the uterus.

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PHARMACOLOGY

THE EFFECT OF A NEUROLYTIC MIXTURE ON CERTAIN BIOCHEMICAL BLOOD INDICES IN TRAUMATIC AND ANAPHYLACTIC SHOCK

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In the prophylaxis and treatment of traumatic shock extensive use has been made recently of various chemical substances with diversity of activity, in the form of neurolytic mixtures, in addition to other measures such as blood, heterogenic protein and synthetic blood substitutes. The use of neurolytic mixtures is based on the fact that, while they act mainly on the central nervous system, they at the same time effect a pharmacological block of the autonomic nervous system, and this prevents the excessive liberation in shock of adrenalin, acetylcholine, histamine and histamine-like substances, thereby decreasing the severity of the reaction of the body [14]. Furthermore, certain drugs included in the lytic mixture affect the carbohydrate metabolism [9]. As investigations have shown [6, 10, 11, 12], in traumatic shock considerable changes are observed in the biochemical blood indices (a fall in the alkali reserve, a lowering of cholinesterase activity and a rise in the blood sugar).

In view of the fact that, after administration of heterogenic protein blood substitutes, complications of an anaphylactic character could arise, we made a comparative study of the effect of a neurolytic mixture on certain biochemical blood indices during the prophylaxis of anaphylactic shock and during the treatment of traumatic shock complicated by anaphylactic shock.

EXPERIMENTAL METHOD

For this purpose we carried out three series of experiments on 30 rabbits. In the first, control, series we studied the biochemical changes in the blood in anaphylactic shock without the use of a neurolytic mixture. In the second series the biochemical changes in the blood were studied with the use of a neurolytic mixture for the prevention of anaphylactic shock. In the third series the biochemical changes in the blood of rabbits were studied during the treatment of traumatic shock, complicated by anaphylactic shock, with a neurolytic mixture.

In the first series of experiments sensitization was induced by giving two subcutaneous injections of 1 ml of ox serum at intervals of 7-10 days. From 10 to 15 days after the second injection of antigen, the experimental animals were used in the main experiment. Anaphylactic shock was caused by the intravenous injection of an assaulting dose (3 ml) of ox serum. The changes in the arterial pressure and in respiration were recorded on the smoked frum of a kymograph by the usual method. Before the experiment and after the intravenous injection of the assaulting dose of antigen determinations were made of the sugar level in whole blood by the Hagedorn-Jensen method, the alkali reserve of the plasma by Van Slyke's method, and the cholinesterase activity by the methods of Zubkova and Pravdich - Neminskaya. Blood for these tests was taken, in a volume of 5 ml, from the carotid artery.

In order to prevent anaphylactic shock we used the following neurolytic mixture: chlorpromazine (2%, 0.5 ml), pentamine (2%, 0.5 ml), benadryl (2%, 0.5 ml) and promedol (2%, 0.5 ml). Before the intravenous injection of the neurolytic mixture the initial levels of the blood sugar, the alkali reserve and the cholinesterase activity were determined. These were subsequently determined 10 minutes, and in some experiments 20 minutes,

TABLE 1

Changes in the Levels of the Blood Sugar, Alkali Reserve and Cholinesterase Activity in Anaphylactic Shock

Type of test	Experiment No.	Initial values	After inject. of antigen	Difference from initial values	Difference (in %)	During shock	Difference from initial values	Difference (in %)	Result
Blood sugar, in mg %	1	120	149	+29	+24	Died			
	2	88	142	+54	+61	172	+84	+95	Died
	3	92	135	+43	+46	171	+79	+85	Died
	5	121	136	+15	+12	Died			
	14	165	246	+81	+49	Died			
Mean		117	161	+44	+37	171	+81	+90	
Alkali reserve of the plasma, in volumes %	1	41,4	30,9	−10,5	−25	Died			
	2	37,3	28,3	−9,0	−24	22	−15,3	−41	Died
	3	41,2	35,4	−5,8	−14	24,9	−16,3	−39	Died
	5	34,2	34,2	0	0	Died			
	14	21,4	15,7	−5,7	−27	Died			
Mean		35,1	28,9	−6,2	−17	23,4	−15,8	−40	
Cholinesterase activity of the plasma, in cm^3 of 0.01 N NaOH solution	1	0,31	0,17	−0,14	−45	Died			
	2	0,38	0,19	−0,19	−50	No determination possible			
	3	0,26	0,18	−0,08	−33	0,14	−0,12	−53%	Died
	5	0,36	0,2	−0,16	−44	Died			
	14	0,18	0,08	−0,1	−55	Died			
Mean		0,3	0,16	−0,14	−46				

after the intravenous injection of the neurolytic mixture, and again 5-10 minutes, and in some experiments 30 minutes, after the intravenous injection of the assaulting dose of antigen. In the third series traumatic shock was induced by repeated stimulation of the sciatic nerve with the electric current from an induction coil, the distance between the primary and secondary coils of which was 5-6 cm. The stimulation lasted 2 minutes and the interval between stimulation was 2-5 minutes. After stimulation of the sciatic nerve, the animal was bled to the extent of 1 % of the body weight (15-20 ml). The neurolytic mixture was injected intramuscularly and intravenously 15 minutes after the end of stimulation of the sciatic nerve, when the arterial pressure had fallen persistently by 40-50% of its initial level and showed no tendency to rise, and also other features of traumatic shock were observed. From 10 to 15 minutes after injection of the neurolytic mixture the assaulting injection of antigen was given. In 5 experiments, before giving the mixture, physiological saline and 5% glucose solution were injected intravenously in a volume of 15-20 ml. Blood for biochemical investigation was taken from the carotid artery before the experiment, after the end of stimulation of the sciatic nerve, after injection of the neurolytic mixture and after intravenous injection of the assaulting dose of ox serum.

EXPERIMENTAL RESULTS

Intravenous injection of the assaulting dose of antigen was accompanied by the development of anaphylactic shock with a lethal outcome. The arterial pressure at the moment of injection of the serum and immediately after the end of the injection was raised, the respiration rate was quickened considerably, and the general behavior of the experimental animal was quiet. From 1 to 2 minutes after the end of the injection of antigen the arterial pressure rapidly (in the course of 3-5 minutes) fell to zero, respiration became slower and more superficial and finally ceased, contractions of the heart being observed for a further 3-5 minutes.

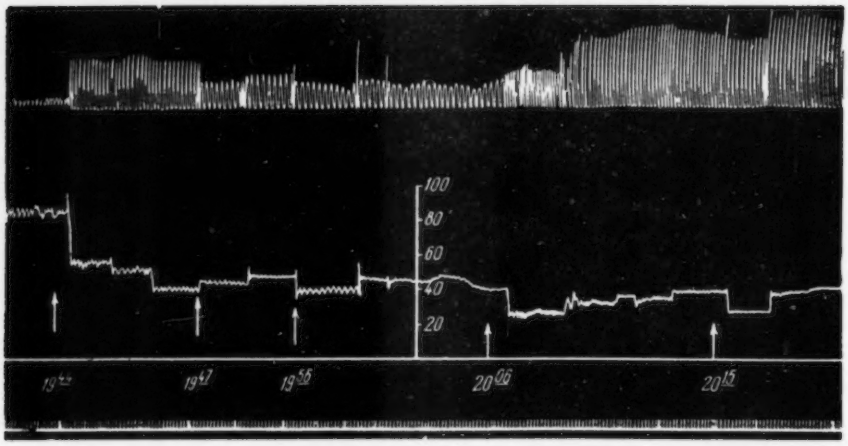

Fig. 1. Changes in the arterial pressure and respiration during prophylaxis of anaphylactic shock by the intravenous injection of a neurolytic mixture. 19 hours 44 minutes: blood taken for biochemical tests and injection of neurolytic mixture. 19 hours 56 minutes: blood taken for biochemical tests. 20 hours 06 minutes: intravenous injection of assaulting dose of ox serum. 20 hours 15 minutes: blood taken for biochemical tests. 20 hours 30 minutes: blood taken for biochemical test and end of experiment. Significance of the curves (from above down): respiration, arterial pressure, zero line, time marker.

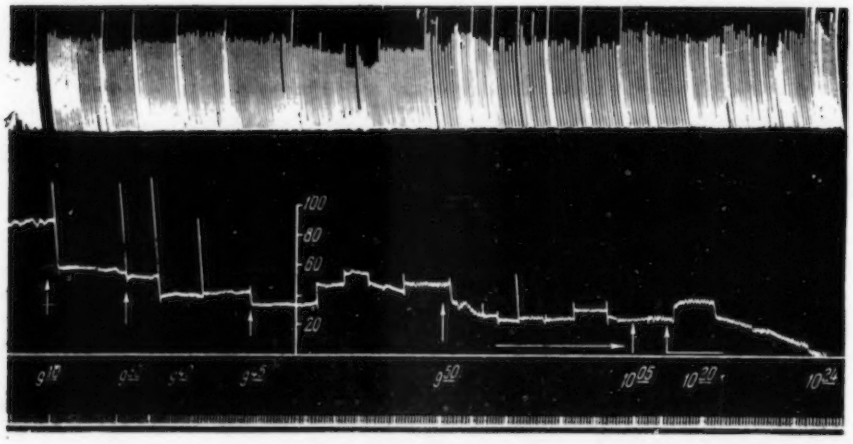

Fig. 2. Change in the arterial pressure and respiration in anaphylactic shock developing on a background of traumatic shock. 9 hours 10 minutes: bleeding (↑), 9 hours 30 minutes - 9 hours 40 minutes: stimulation of the sciatic nerve (↑), 9 hours 45 minutes: blood taken for biochemical tests (↑), 9 hours 45 minutes - 9 hours 49 minutes: intravenous injection of physiological saline (30 ml), 9 hours 50 minutes - 10 hours 05 minutes: intravenous injection of neurolytic mixture (↔), 10 hours 20 minutes: intravenous injection of assaulting dose of antigen (5 ml), 10 hours 24 minutes: death of rabbit. Significance of the curves (from above down): respiration, arterial pressure, zero line, time marker.

It will be seen from the findings in Table 1 that, in anaphylactic shock, the alkali reserve fell by between 17-40% and the cholinesterase by an average of 46%, while the blood sugar rose by 37-90% of its initial level. These changes were the greater the more severe the course of the anaphylactic shock. The indices showing the

TABLE 2

Changes in the Levels of the Blood Sugar, Alkali Reserve and Cholinesterase Activity after Injection of a Neurolytic Mixture

Type of test	Experiment No.	Initial values	After inject. of mixture		Difference from initial values, in %	After inject. of antigen		Difference from initial values, in %	
			after 10 min	after 20 min		after 10 min	after 30 min		
Blood sugar (in mg%)	6	172	231	—	+34	237	—	+37	Lived
	7	94	114	—	+21	140	—	+48	Lived
	13	87	118	—	+35	112	112	+28	Lived
	15	96	568	—	+491	Died			
	16	138	155	129	+1	150	—	+17	Lived
	17	128	140	129	+1	150	—	+17	Lived
	18	398	407	—	+2	Died			
	21	101	149	152	+50	190	—	+86	Lived
	22	135	146	128	—5	193	198	+46	Lived
Mean . . .		108	186	167	+43	184	142	+67	
Alkali reserve, in volumes %	6	42,4	42,4	—	0	Could not be determined			
	7	38,5	53,8	—	+39	51	—	+32,4	Lived
	13	39,5	42,4	—	+7,3	43,3	38,5	—2,5	Lived
	15	24,2	22,3	—	—7,8	Died			
	16	52,8	51,9	—	—1,7	43,3	—	—17	Lived
	17	41,4	48,1	48,1	+16	27,1	—	—36,4	Lived
	18	29	29	—	0	Died			
	21	46,2	42,4	49	+6	39,5	—	—14,4	Lived
	22	—	37,5	—	—	26		—30,6	Lived
	23	36,6	42,4	44,3	+21	40,4	30	—18	Lived
Mean		42,5	45,1	47,1	+14	38,6	34,2	—10	
Cholinesterase activity, in cm^3 of 0.01 N NaOH solution	6	0,18	0,24	—	+30	0,21	—	+5	Lived
	7	0,23	0,27	—	+17,3	0,13	—	—43,4	Lived
	13	0,27	0,26	—	—4,4	0,15	0,14	—48	Lived
	15	0,13	0,08	—	—37	Died			
	16	0,27	0,27	—	0	Could not be determied			
	17	0,19	0,19	0,19	0	0,18	—	—5	Lived
	18	0,24	0,2	—	—16,6	Died			
	21	0,36	0,33	0,19	—46	0,19	—	—46	Lived
	22	0,25	0,21	0,22	—12	0,17	0,17	—32	Lived
	23	0,23	0,22	0,2	—13	0,2	0,2	—13	Lived
Mean		0,25	0,25	0,2	—17	0,18	0,17	—31	

greatest changes in the blood in shock were those of the cholinesterase activity, for they were recorded at times when the other indices were unchanged or were changed only insignificantly (rabbit No. 5).

Intravenous injection of a neurolytic mixture for prophylaxis of anaphylactic shock led to a fall in the arterial pressure by 50-60% of its initial value, and the more rapidly the mixture was injected, the greater the fall in the pressure. Two of the rabbits died during rapid injection. Immediately after injection of the mixture the respiration rate increased, and 2-3 minutes later it became slow (10-12 respirations per minute) and superficial, with a decrease in the amplitude of the respiratory movements.

TABLE 3

Changes in the Levels of the Blood Sugar, Alkali Reserve and Cholinesterase Activity during Traumatic Shock

Type of test	Experiment No.	Initial level	Traumatic shock	Difference, in %	Injection of mixture	Difference from initial value, in %	Injection of antigen	Difference from initial value, in %	Result
Blood sugar, in mg %	8	86	140	+62	147	70	154	+78	Died
	9	96	186	+93	294	206	306	+216	Died
	10	193	281	+45	376	94	Died		
	11	117	215	+83	185	+58	181	+54	Died
Mean		123	205	+66	250	+103	214	+65	
Alkali reserve, in volumes %	8	35,7	12,8	—65	9,9	—72	6,6	—78	Died
	9	24	14	—41	14	—41	Could not be determined		
	10	26,2	9,9	—63	12,8	—54	Died		
	11	30	6,9	—77	14,7	—51	15,7	—41	Died
Mean . . .		29	10,9	—62	12,8	—55	11,1	—59	
Cholinesterase activity of the plasma in cm^3 of 0.01 M NaOH solution	8	0,51	0,36	—29	0,35	—31	0,18	—65	Died
	9	0,35	0,33	—5,7	0,26	—26	0	—100	Died
	10	0,35	0,35	0	0,12	—65	Died		
	11	0,25	0,24	—4	0,12	—52	0,03	—88	Died
Mean		0,36	0,31	—13	0,21	—14	0,1	—84	

The intravenous injection of the assaulting dose of ox serum was accompanied by a doubling of the respiration rate in the course of 10-12 minutes, and subsequently it remained rather high until the end of the experiment, being higher than after the injection of the neurolytic mixture but lower than before the drugs were given.

After a brief rise, the arterial pressure fell in response to the injection of the assaulting dose of antigen rather below the level which was found 10 minutes after injection of the mixture and at the end of the experiment it rose slowly to 60-70 mm (Fig. 1).

All the experimental animals of this series tolerated satisfactorily the intravenous injection of the assaulting dose of ox serum, and on the following days their behavior was indistinguishable from normal.

In Table 2 are shown the changes in certain biochemical blood indices which took place after injection of a neurolytic mixture for prophylaxis of anaphylactic shock.

In this series of experiments the blood changes determined by means of the biochemical tests arose both under the influence of the neurolytic mixture and of the antigen. In rabbit No. 15, the blood sugar rose by almost 500% in the course of 2-3 minutes, the cholinesterase activity fell by 37% and the alkali reserve was almost unchanged. In rabbit No. 18 the variations in these indices were minimal.

After 10 minutes, the slow intravenous injection of the neurolytic mixture caused an increase in the blood sugar of 72% on the average, but after 20 minutes the level fell, although it still exceeded the initial level by 43% on the average.

The assaulting dose of ox serum caused a moderate increase in the blood sugar by comparison with the level found in the previous determinations, although the mean figures were unchanged. The alkali reserve of the plasma increased in all the experiments under the influence of the neurolytic mixture by an average of 6.1%

after 10 minutes and by 14% 20 minutes after injection of the mixture. Injection of the antigen lowered this level in some cases only when compared with the maximal figures established after injection of the preparations, and it stayed above the original values, falling by an average of 9.1-10.1% of the initial value.

After injection of the mixture the variation in the cholinesterase activity took the form of either an insignificant increase or decrease. Twenty minutes after injection the cholinesterase activity fell very moderately in relation to the previous determination and by 25.5-31.0% in relation to its initial value.

Traumatic shock, as already pointed out, was caused by stimulation of the sciatic nerve with an electric current after preliminary bleeding. The bleeding itself led to a fall in the arterial pressure, on the average by 18 mm. Under these circumstances, in some experiments the respiration rate increased by 4-10 per minute, while in others it remained slower.

After bleeding, traumatic shock developed after 5-10 stimulations of the sciatic nerve. The arterial pressure fell by 30-40 mm from the level established after bleeding, the corneal reflex became sluggish and respiration became embarrassed, and moreover, in some experiments it was rapid, whereas in others, after a period of increased rate, respiration became slow and embarrassed, especially the phase of inspiration. When the picture of traumatic shock was fully developed, the intravenous injection of the neurolytic mixture led to a catastrophic fall in the arterial pressure (in 5 rabbits to zero) and to death of the animals within the next 5 minutes. In 6 experiments the pressure after injection of the mixture settled at between 18 and 25 mm Hg. In one case death from traumatic shock took place even before injection of the mixture.

The subsequent (10-15 minutes after injection of the mixture) intravenous injection of the assaulting dose of antigen led to a rapid fall in the arterial pressure to zero and to death of the experimental animals within 3-5 minutes. The intravenous infusion of 5% glucose solution and physiological saline, in volumes of 20-30 ml, both before injection of the neurolytic mixture in the course of development of traumatic shock and together with the mixture after stimulation of the sciatic nerve, did not prevent death of the animals from the intravenous injection of the assaulting dose of ox serum (Fig. 2).

The results relating to the changes in the blood sugar, alkali reserve and cholinesterase activity are shown in Table 3.

As may be seen from the figures in Table 3, the onset of traumatic shock was characterized by a rise in the blood sugar and a fall in the alkali reserve. The cholinesterase activity fell moderately, on the average by 13%.

Under the influence of the intravenous injection of the neurolytic mixture the blood sugar rose and the variations in the alkali reserve took the form of both a decrease and an increase. This feature must evidently be interpreted not as a true increase in the alkali reserves of the blood, but as the result of an increase in the carbon dioxide, due to the slowing of the respiration after injection of the neurolytic mixture, which essentially lowered the ventilation volume of the lungs and the sensitivity of the respiratory center. It must also be remembered that there was a possibility of gaseous acidosis on account of stasis of the blood in the capillaries in association with the lowered velocity of the blood flow.

The injection of the neurolytic mixture into rabbits in a state of traumatic shock caused, in contrast to control experiments, a marked fall of 41% in the cholinesterase activity.

After injection of the assaulting dose of antigen all the rabbits died from anaphylatic shock. In relation to the blood sugar a tendency for this to fall was observed, from 250 to 214 mg%, which could evidently be accounted for by exhaustion of the glycogen reserves of the liver. The value of the alkali reserve before death of the experimental animal fell by 41% by comparison with the initial values, and rose slightly in relation to the minimum level observed during the experiment (on the average from 6.9 to 15.7 volumes %).

SUMMARY

The biochemical changes occurring in the blood (blood sugar level, alkali reserve, cholinesterase activity) were insignificant when the neurolytic mixture (2% solutions of chlorpromazine, pentamine, promedol and benadryl) was employed for preventing anaphylactic shock.

The administration of this mixture in the instance of a fully developed traumatic shock not only does not prevent the development of anaphylactic shock, but even aggravates both the course and the outcome of the

traumatic shock. This is confirmed by the biochemical changes in the blood: in comparison with the changes observed in experiments on the prophylaxis of anaphylactic shock with the neurolytic mixture, blood sugar rose 2.4 times, the alkali reserve dropped 5 - 5.9 times and cholinesterase activity fell 2.4 - 2.7 times.

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THE EFFECT OF ETHYZINE* ON THE CONDITIONED REFLEXES OF DOGS

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Neurolytic drugs of the phenothiazine group have recently been widely used for purposes of potentiation of anesthesia and the production of hypothermia [3, 7].

After chlorpromazine and phenergan, one of the more potent members of this groups is ethyzine (synonyms: phenethazine, anergan, lisergin), which, according to the investigations of M. D. Mashkovskii and his co-workers [10], possesses antihistamine and adrenolytic actions. According to I. E. Kovalev [6], ethyzine depresses the electrical activity of the cerebral cortex.

The character of the effect of ethyzine on conditioned reflexes has not so far been studied, whereas a large number of investigations relate to chlorpromazine [1, 2, 4, 5, 12].

In view of the topical importance of this question, in our present research we set out to study the effect of ethyzine on conditioned reflexes in dogs.

EXPERIMENTAL METHOD

The investigation was carried out on 4 dogs in a conditioned reflex chamber. The dogs Valetka and Laska had conditioned reflexes to food, with short periods of delay (10 seconds), from various analyzers, and differentiation of one reflex. In Laska, besides short-delay reflexes to light, a conditioned reflex to a knocker, with a delay of 2 minutes, was developed. As unconditioned stimulus we used a meat and biscuit powder (10g). Registration of the saliva was done by the method of A. I. Makarychev [9]. At the same time, the respiratory and masticatory movements and also the stimulus and time markers were recorded on the kymograph.

In two other dogs Sil'va and Sedaya, protective respiratory conditioned reflexes were developed to a bell as positive stimulus, a bell as differential, to light and to touch. The unconditioned stimulus was ammonia, which was expelled from a system of tubes into the nostril of the animal. All the conditioned stimuli except the light were delayed for 5 seconds from the beginning of action of the unconditioned stimulus, and the light was delayed for 30 seconds.

Ethyzine was injected intramuscularly as a 1% solution in distilled water, in doses of 0.5 to 25 mg/kg body weight. The investigation of the conditioned reflexes began 15 minutes after injection of the drug, and continued for 3-6 hours.

Altogether 35 experiments with ethyzine and 6 control experiments, in which distilled water was injected intramuscularly, were carried out.

* Ethyzine is N-(2-dimethylaminoethyl)-phenothiazine hydrochloride, or phenethazine.

TABLE 1

The Effect of Ethyzine on Conditioned Reflexes in the Dog Valetka

Experiment No.	1	2	3	4	5	6
Dates of experiments	3/1-57	7/1-57	9/1-57	17/1-57	21/1-57	24/1-57
Doses of ethyzine, in mg/kg body weight	0,5	1	2	5	7	10
Metronome +240	8 10 9	10 5 8	9 0 0	9 0 5	7 0 3	8 0 0
Light	2 4 2	8 0 1	4 0 2	4 0 0	5 0 0	5 0 0
Touch	4 5 5	7 1 2	7 0 2	5 0 0	5 0 0	4 0 0
Metronome -60	0 1 0	2 0 0	0 0 1	1 0 5	0 0 0	2 0 0
Total	14 20 16	27 6 11	20 0 5	19 0 10	17 0 3	19 0 0

Note. The magnitude of the conditioned reflexes is given in drops of saliva during 30 seconds of action of the conditioned stimuli. In each experiment the first column of figures gives the initial magnitude of the reflexes, the second gives the magnitude of the reflexes 20-60 minutes after administration of ethyzine and the third gives the magnitude of the reflexes 3-4 hours after administration of ethyzine.

EXPERIMENTAL RESULTS

The observations showed that a small dose of ethyzine (0.5 mg/kg) caused disturbance of the cortical processes. Under these circumstances the positive conditioned reflexes were increased and differentiation was disinhibited. In Valetka, the magnitude of the conditioned reflexes was increased by 1-2 drops of saliva during 30 seconds of action of the stimulus, and in Laska by 2-5 drops. Differentiation in the first dog was disinhibited up to one drop of saliva, and in the second to 2 drops. In Laska, besides disinhibition of differentiation, a shortening of the inhibitory phase of the delayed conditioned reflex was observed (Tables 1 and 2).

The increase in the magnitude of the positive conditioned reflexes with, at the same time, disinhibition of differentiation and shortening of the inhibitory phase of the delayed reflex indicated weakening of the processes of internal inhibition. Simple observation of the animals' behavior meanwhile showed no abnormality after administration of small doses of ethyzine.

Increasing the dose of ethyzine to 1 mg/kg caused in Valetka, a sharp fall in the conditioned reflexes to the metronome, to touch and, as far as complete extinction, to light. Differentiation was not disinhibited. A similar picture was observed in the case of Laska. In the latter, however, the action of ethyzine developed gradually, so that the changes in the cortical processes could be followed. In the first 30 minutes after administration of ethyzine, disappearance of the delayed conditioned reflex to the knocker and dishinhibition of differentiation to light up to 3 drops were observed (experiment No. 2, Table 2). The magnitude of the positive conditioned reflex to light was not altered under these circumstances.

The fact that there was marked disinhibition of differentiation to light and no change in the magnitude of the positive conditioned reflex to the same stimulus indicated considerable weakening of internal inhibition. Meanwhile, the complete extinction of the delayed conditioned reflex was evidence of a lowering of the level of excitation in the cerebral cortex. In the 30 minutes following administration of ethyzine (1 mg/kg) gross weakening of excitation was observed – the conditioned reflex to light disappeared and differentiation was not disinhibited (Table 2).

After larger doses of wthyzine were given (from 1.5 to 2.0 mg/kg), in Valetka the artificial conditioned reflexes fell to zero and differentiation also had a value of zero (Table 1). In Laska, the action of these doses

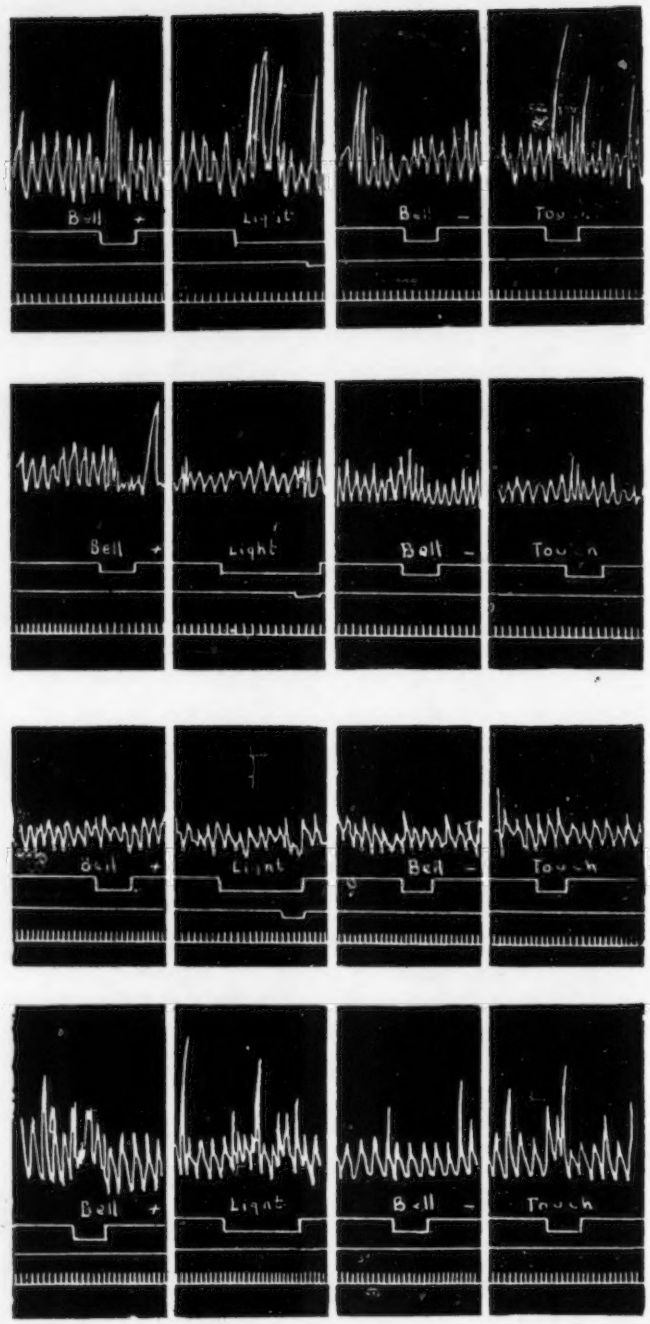
TABLE 2

The Effect of Ethyzine on Conditioned Reflexes in the Dog Laska

Experiment No.	1			2				3			4			5			6		
Dates of experiments	21/XII 1956			24/XII 1956				26/XII 1956			29/XII 1956			2/I 1957			5/I 1957		
Doses of ethyzine, in mg/kg body weight	0,5			1				1			1,5			1,5			2		
Metronome	0002	0022	0002	0012	0000	0000	0012	0001	0013	1024	0002	0002	0004	0104	0016	1004	0004	0010	0002
Light +	3	8	4	4	4	0	3	6	4	3	4	2	4	7	3	5	3	6	0
Light -	0	2	0	1	3	0	1	1	1	1	0	2	0	3	0	1	1	1	1
Total	5	14	6	8	7	0	6	8	9	11	6	6	8	15	10	11	8	8	3

Experiment No.	7			8			9			10			11			12		
Dates of experiments	15/I 1957			18/I 1957			21/I 1957			24/I 1957			28/I 1957			30/I 1957		
Doses of ethyzine, in mg/kg body weight	3			5			7			10			15			25		
Metronome	0002	0014	0002	0003	0000	0010	0046	0000	0001	0026	0000	0001	0014	0000	1001	0004	0001	0010
Light +	4	2	4	5	0	1	3	0	0	5	1	0	4	0	0	2	0	1
Light -	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	0
Total	6	7	6	8	0	2	14	0	1	13	1	2	9	0	3	6	1	2

Note. The delayed conditioned reflex to the metronome is given in drops of saliva during each 30 seconds of action of the stimulus (for 2 minutes altogether). The magnitude of the conditioned reflex to light and of differentiation are also given in drops of saliva in 30 seconds of action of the stimuli. The remaining explanatory notes are as in Table 1.



The effect of ethyzine on the conditioned defensive respiratory reflexes in the dog Sil'va. Significance of the curves (from above down): pneumogram, conditioned stimulus marker, unconditioned stimulus marker, time marker (3 seconds). Legend: a) Before injection of ethyzine; b) 40 minutes after injection of 3 mg/kg of ethyzine; c) 40 minutes after injection of 10 mg/kg of ethyzine d) on the 4th day after injection of ethyzine.

(1.5-2.0 mg/kg) was less pronounced than that of the lower dose (1 mg/kg) (Table 2). This fact was evidence that the dog was becoming habituated to the doses of ethyzine which we were using.

In subsequent experiments, in order to avoid the development of the habituation factor, the successive increase of the doses of ethyzine was carried out more quickly.

When the dose of ethyzine was increased to 5 mg/kg, changes were observed in the general behavior of the animals. The reactions to their names and to the sight of the experimenter were diminished. When not on the apparatus they lay down more, and when standing and walking they staggered and moved their paws clumsily, although they jumped up on to the bench quite independently.

Investigation of the conditioned reflexes under these circumstances showed a sharp diminution of excitation. The conditioned reflexes to all the stimuli completely disappeared in both dogs, and differentiation was not disinhibited in 5 to 7 tests, and in the remaining two it was disinhibited by one drop of saliva.

On increasing the doses of ethyzine to 7, 10, 15 and 25 mg/kg the conditioned reflexes fell to zero in the first 30 minutes after administration of the drug, and were absent throughout the whole period of observation (3-6 hours). At the same time the dogs became sluggish and reacted feebly to external stimuli. Refusal to eat was not observed in any of the experiments.

It must be pointed out that ethyzine, in the doses which we used, besides leading to extinction of the salivatory conditioned reflexes, caused a parallel fall in the magnitude of the unconditioned salivatory reflex to a portion of meat and biscuit powder from 40 to 32 drops on the average in Valetka and from 56 to 47 drops in Laska. The fall obtained in response to doses of ethyzine from 7 to 25 mg/kg was especially marked.

The rapid extinction of the salivatory conditioned reflexes with the action of comparatively small doses of ethyzine (1 mg/kg) did not enable the course of the changes in the cortical processes with large doses of the drug to be studied. Accordingly, as an index of the higher nervous activity, we used the conditioned defensive respiratory reflexes, which have been found in our laboratory to be more resistant to the action of chlorpromazine than the salivatory conditioned reflexes.

The experiments showed that ethyzine, in a dose of up to 2 mg/kg, did not affect the defensive respiratory reflexes. Doses of ethyzine of 3-5 mg/kg gave an appreciable effect in both dogs — the conditioned reflexes to the bell and touch were severely weakened and the conditioned reflex to light disappeared altogether. With these doses, differentiation was disinhibited. On increasing the dose of ethyzine to 7 mg/kg, in addition to the disturbances already mentioned, extinction of the conditioned reflex to touch was observed. Disappearance of the conditioned reflex to the bell, as a strong stimulus, took place when the dose of ethyzine reached 10 mg/kg. In the following experiments, when higher doses (12-15 mg/kg) of this drug were given, the disturbance of the respiratory conditioned reflexes was less pronounced on account of habituation of the dogs (see figure). The unconditioned defensive respiratory reflex to ammonia was sharply diminished by large doses of ethyzine (10-15 mg/kg) but did not disappear completely.

The rate of recovery of the conditioned reflexes after ethyzine depended on the dose of the drug, on the type of conditioned reflex and on the degree of habituation of the dogs. Recovery of the salivatory conditioned reflexes with a dose of ethyzine of 0.5 mg/kg took place 3-4 days after its administration, and with doses of 1-3 mg/kg within the first 24 hours. During the recovery period, paradoxical and ultraparadoxical hypnotic phases appeared; After doses of ethyzine of 5-7 mg/kg, the conditioned reflexes were restored to normal on the third day. With higher doses (10-15 mg/kg) recovery was prolonged to 4-5 days.

Recovery of the defensive conditioned respiratory reflexes followed a more rapid course, being completed with doses of ethyzine of 3-5 mg/kg in 3-4 hours, and after 10-15 mg/kg during a period of 3 days.

It must be pointed out that in control experiment in which distilled water was injected intramuscularly into dogs, their conditioned reflex activity was unchanged.

Analysis of the results described above shows that the disturbance of the function of the cerebral cortex in dogs develops under the influence of a comparatively small dose of ethyzine (0.5 mg/kg). This dose has a selective effect on the process of internal inhibition and evidently does not affect the process of excitation. Internal inhibition is weakened, causing disinhibition of differentiation, shortening of the inhibitory phase of the delayed conditioned reflex and also an increase in the magnitude of the conditioned reflexes to positive stimuli. On increasing the dose of ethyzine to 1 mg/kg, besides weakening of the internal inhibition, a decrease in the strength of the process of excitation takes place. The positive conditioned reflexes are considerably diminished or completely extinguished.

On increasing the doses of ethyzine further, to 3-25 mg/kg, the weakening of the cortical processes increases progressively, which leads to complete extinction not only of the salivatory reflexes but also of the more stable defensive conditioned respiratory reflexes to reinforcement with ammonia. Changes in the unconditioned salivatory and respiratory reflexes take place with higher doses of ethyzine (1-10 mg/kg) than in the conditioned reflexes (0.5-3.0 mg/kg).

As we know, the study of the mechanism of action of anesthetics and hypnotics on the functions of the central nervous system has shown that anesthetics cause primary weakening of the higher form of cortical inhibition, and then lower the level of the process of excitation [8, 11, 13]. These changes in the activity of the cerebral cortex develop in response to doses of anesthetics and hypnotics which are not sufficiently large to cause disturbances of subcortical functions.

Comparison of these findings with the results of our investigation shows that the initial effect of ethyzine on the function of the cerebral cortex is similar to the action of anesthetics.

SUMMARY

The author studied the effect of ethyzine on the salivatory food and protective respiratory conditioned reflexes in 4 dogs. There is evidence that the disturbance of the cerebral cortex function sets in when ethyzine is administered in a dose of 0.5 mg/kg. This weakens the process of internal inhibition but has no effect on the excitation process. Positive conditioned reflexes become increased, differentiation — disinhibited, while the inhibitory phase of the delayed reflex is shortened. 1-25 mg/kg of ethyzine provokes a progressive weakening of the cortical processes with the conditioned reflexes dropping to zero. The conditioned reflexes become less intense when higher doses of the preparation (1-10 mg/kg) are given.

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THE EFFECT OF CORTISONE AND GLUTAMINIC ACID ON THE EXCITATION OF THE NERVOUS SYSTEM IN RATS

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The therapeutic action of cortisone is known to be often accompanied by a number of side effects, one of which is an increase in the excitation of the nervous system. In patients receiving cortisone for a long time, mild euphoria and insomnia and — more rarely — psychoses may develop.

These clinical findings of a change in the excitation of the nervous system under the influence of cortisone were confirmed by animal experiments [5] in which it was shown that this hormone lowers the threshold of electric shock in rats and also disturbs the central nervous activity of dogs [1].

In a previous paper [2] we showed that the administration of cortisone to rats is accompanied by accumulation of ammonia in the brain, and for this reason the hypothesis was put forward that the increased excitation of the central nervous system under the influence of the hormone was associated with the increased concentration of NH_3 in the brain tissue. It was shown in the same paper that administration of glutaminic acid at the same time as the cortisone lowers the concentration of this toxic product.

The aim of the present investigation was to find out whether a change in the excitation of the nervous system takes place under the influence of cortisone if, at the same time, glutaminic acid is administered, thereby preventing the accumulation of ammonia.

EXPERIMENTAL METHOD

Experiments were carried out on male white rats weighing 180-200 g. Cortisone was injected intramuscularly in a dose of 2.5-5.0 mg daily, and neutralized L-glutaminic acid subcutaneously in a dose of 100 mg daily.

In determining the degree of excitation by the duration of sleep, the rats were given intraperitoneal injections of sodium amytal solution in a dose of 8 mg/100g body weight. Measurement of the time began from the moment when the animal stopped trying to turn over from the supine to the prone position. Sleep was considered to have ended when the rat turned to the prone position.

In determining the degree of excitation by the magnitude of the threshold of electric shock, we used an apparatus assembles in our Institute along the lines suggested by Woodbury [4]. This apparatus enables the current strength and duration of action to be accurately controlled.

The magnitude of the threshold of electric shock was determined by the method of the author cited above [4]. Stainless steel electrodes were applied to the animal's ears and a current passed for 0.6 seconds. In the measurement of the threshold of electric shock an accuracy of 0.25 ma was attained.

TABLE 1

The Effect of Cortisone and Glutaminic Acid on the Duration of Sleep in Rats (in minutes)

Animal No.	Control	Cortisone	Cortisone + glutaminic acid
1	95	32	65
2	60	30	48
3	85	43	65
4	46	20	33
5	85	35	63
6		40	62
7		38	
Mean	74	34	56

Usually in the course of the two weeks before the start of the experiment, 3-4 determinations of the threshold of electric shock were made in the rats. This value was taken as the control level, and animals which did not give more or less constant values in the control period were excluded from the experiments. Animals were subjected to the action of the electric current not less than 2-3 days after the preceding experiment.

EXPERIMENTAL RESULTS

It was shown in preliminary experiments that after administration of cortisone, the sleep of the rats became shorter and more restless (Table 1). The simultaneous administration of glutaminic acid increased the duration of sleep, although not to normal. Whereas the sleep period in the control rats lasted an average of 74 minutes, and cortisone decreased it to 34 minutes, after administration of glutaminic acid the animals' sleep lasted 56 minutes.

Having obtained results which showed that glutaminic acid to some degree prevented the increase in the excitation of the nervous system due to cortisone, we decided to verify and to augment these findings by determining the degree of excitation by a more accurate method , by the magnitude of the threshold of electric shock.

Three series of experiments were carried out. In each series, at the end of the control period the animals were divided into three groups. The rats in the first group received 0.2 ml of 0.9% NaCl solution daily, the second group 2.5-5.0 mg of cortisone daily and the third 100 mg of glutaminic acid as well as the hormone daily. It must be pointed out at once that the changes in the threshold of electric shock in the control rats of all three series were insignificant, amounting to only 2-3% at the end of the experiment, and they will not, therefore, be considered further.

The first series of experiments was conduced on 12 animals, 4 in each group. It can be seen from Fig. 1 that cortisone increased the excitation of the nervous system, as shown by a lowering of the threshold of electric shock, which reached 28% on the 15th day of the experiment. The administration of glutaminic acid together with with cortisone to the other group of rats almost completely neutralized the action of the hormone on the level of excitation during the first week, but in these rats a fall in the threshold of electric shock subsequently took place, so that it amounted to 24% at the end of the experiment.

The second series of experiments was performed on 34 animals. As shown in Fig. 2, administration of cortisone in a dose of 5 mg daily lowered the threshold of electric shock on only the third day, by an average of 30%. On the 9th day of administration, the decrease was 20%, and after 12 days it reached 30% once again. The sharp change in the threshold of electric shock in the first 3 days may be accounted for by a rise in the air temperature to 30° , whereas during the whole course of the experiment the temperature varied between 15 and 20° .

In order to ascertain the effectiveness of glutaminic acid during such intensive changes in excitation, the rats were divided into two subgroups. One subgroup continued to receive cortisone, and the other received glutaminic acid together with the cortisone. As may be seen from the figures in Table 2, in the first subgroup the fall in the threshold of electric shock continued and reached 36%, whereas in the second subgroup no further increase in excitation took place.

The other group of rats received glutaminic acid in addition to cortisone from the beginning of the experiment, and this somewhat prevented an increase in excitation. For instance, the fall in the threshold of electric shock after 3 days was only 15% , after 9 days 11% and on the 12th day it reached 15% .

In order to find out the duration of this amino acid, in some of the rats its administration was discontinued, although cortisone continued to be given. After only 2 days the value of threshold of electric shock in these rats

TABLE 2

The Effect of Cortisone (2.5 mg a Day) and Glutaminic Acid on the Threshold of Electric Shock in Rats (threshold values are expressed in milliamperes)*

Animal No.	Preparations administered	Before admonistration				During administration				Remarks
		25/VI	1/VII	4/VII	9/VII	16/VII	19/VII	23/VII	26/VII	
1	0,9% NaCl	8,25	8,75	9,0	9,0	9,25	9,25	9,0	8,75	
2		7,75	8,0	7,75	8,25	8,25	8,0	8,25	8,0	
3		10,5	9,75	10,25	10,5	10,75	10,5	10,5	10,5	
4		9,0	9,25	9,0	9,0	9,0	9,25	9,0	8,75	
5	Cortisone	8,0	9,25	9,5	9,75	8,25	7,5	7,5	8,25	Cortisone + glutaminic acid after July 19
6		7,25	7,0	8,5	8,75	7,25	7,0	8,0	8,0	
7		7,5	7,25	7,75	—	6,5	6,0	6,25	6,0	
8		8,75	9,0	9,0	9,0	7,75	7,0	6,0	6,0	
9		8,25	9,0	9,25	9,25	8,0	7,25	7,0	7,25	
10		7,0	8,5	9,0	9,0	7,75	7,0	6,5	6,25	
11	Cortisone + glutaminic acid	10,0	10,5	9,5	10,5	12,0	11,25	10,0	9,75	Cortisone alone after July 19
12		10,0	10,25	10,5	10,5	12,0	11,0	9,25	9,0	
13		7,75	9,0	8,75	8,75	8,75	9,0	7,0	7,75	
14		8,75	10,0	9,75	10,0	11,0	10,0	10,0	9,25	
15		8,5	8,25	8,75	9,0	10,0	9,5	9,0	8,0	
16				7,25	7,0	7,0	7,5	7,5	6,25	

* Administration of the preparations began on July 12, 1958 .

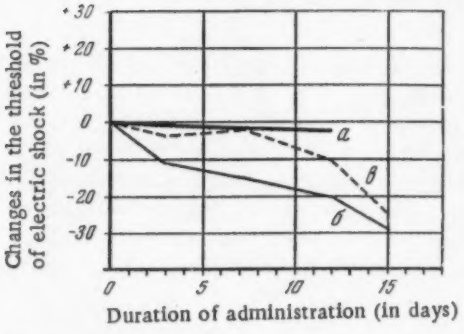

Fig. 1. Changes in the threshold of electric shock under the influence of cortisone (5 mg a day) and glutaminic acid . a) Control b) administration of cortisone; c) administration of cortisone + glutaminic acid .

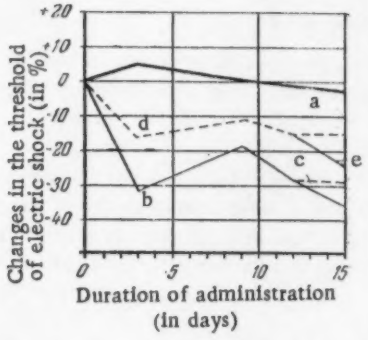

Fig. 2. Changes in the threshold of electric shock under the influence of cortisone (5 mg a day) and glutaminic acid. a) Control; b) administration of cortisone; c) administration of glutaminic acid in addition to cortisone ; d) administration of cortisone + glutaminic acid; e) continued administration of cortisone and discontinuation of glutaminic acid.

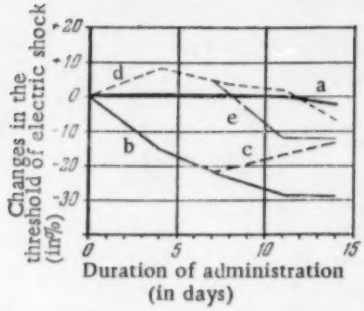

Fig. 3. Changes in the threshold of electric shock under the influence of cortisone (2.5 mg a day) and glutaminic acid. a) Control; b) administration of cortisone; c) administration of glutaminic acid in addition to cortisone; d) administration of cortisone + glutaminic acid; e) continued administration of cortisone and discontinuation of glutaminic acid.

was 10 % below the previous value, whereas in the animals which continued to receive glutaminic acid the excitation was virtually unchanged.

To investigate the relationship between the effectiveness of action of the glutaminic acid and the dose of cortisone, in the third series of experiments the dose of cortisone was reduced to 2.5 mg a day. As may be seen from Table 2 and Fig. 3, on halving the dose of the hormone, the changes in the threshold of electric shock under the influence of cortisone were the same as in the previous experiments. At the same time, the action on the glutaminic acid was more effective with a dose of 2.5 mg a day, for on giving the amino acid to rats hitherto receiving only cortisone an appreciable fall in the level of excitation took place, which was not observed if the rats received a dose of 5 mg of the hormone daily.

When glutaminic acid was given from the beginning of the experiment, the action of cortisone on the excitation of the nervous system was completely neutralized; under these circumstances the value of the threshold of electric shock was even slightly above that that of the controls, and the increase in excitation began only on the 11th day of the experiment.

The results obtained leave no doubt that glutaminic acid prevented the increase in excitation of the nervous system during administration of cortisone. Several suggestions may, however, be made about the mechanism of its action.

It was shown in a previous paper [2] that the administration of cortisone to rats is accompanied by the accumulation of ammonia in the brain, and that glutaminic acid removes this toxic metabolic product. In view of these findings it may be supposed that this factor is responsible for the lowering of the excitation of the brain. However, in spite of the complete normalization of the NH_3 content which was obtained in the above-mentioned work, in our investigation the effectiveness of this amino acid fell toward the end of the experiment, and in one experiment the curve showing changes in the threshold of electric shock was slightly below the control curve after the first few days.

Since the repeated action of an electric current may lead to a number of irreversible changes in nerve tissue it may be postulated that it is this effect which lowered the effectiveness of the glutaminic acid. This hypothesis was overthrown, however, by the experiment in which the criterion of excitation was the length of sleep caused by injection of sodium amytal, since in this case the amino acid only partially restored the length of the period of sleep in the rats. There is no doubt that it is not only ammonia metabolism that is disturbed by cortisone. It is, therefore, a more likely hypothesis that glutaminic acid neutralizes the NH_3 but does not remove the products of certain other changes in metabolism, which gradually accumulate and lead to the increased excitation. These disturbances may, possibly, be connected with changes in the electrolyte balance of sodium and potassium, which is known to be affected by cortisone [3].

SUMMARY

The effect of cortisone and glutaminic acid on the excitability of the nervous system was studied in white rats. The weight of the animals varied from 180 to 200 g. The degree of excitability of the animals was determined by the duration of sodium amytal-induced sleep and by the value of the electric shock threshold (EST). The latter was determined with the aid of an instrument suggested by Woodbury. The results obtained demonstrated that cortisone administration (2.5-5.0 mg per day) was accompanied by a considerable rise of the excitation of the nervous system. With the simultaneous action of the glutaminic acid, the excitability changed insignificantly or not at all. Interruption of the glutaminic acid injections without discontinuance of the hormone administration reduced the EST value sharply.

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THE REACTIVITY OF THE CARDIOVASCULAR SYSTEM IN EXPERIMENTAL NONINFECTIOUS PERITONITIS

COMMUNICATION I. REACTION TO THE INTRAVENOUS INJECTION OF ADRENALIN AND CAFFEINE

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The study of the changes in the general reactivity of the body during the development of a pathological process is of great theoretical and practical importance.

The presence of a pathological process leads to a functional change in the reflex apparatus, which may react differently, under these circumstances, to the action of stimuli. In such cases it may be possible to observe, for example, a change in the response to the intravascular injection of various drugs [7]. Changes in the reactions of the vessels to administration of drugs have also been observed in inflammation [21], in septic febrile diseases [8], in poisoning by enzyme poisons [13], in atherosclerosis [11, 12], in persons resuscitated after clinical death [6], in increased intraperitoneal pressure [9], in different forms of shock [2] and in experimental typhoid fever [4].

Several authors [2, 4, 15-18] account for the changes in the response reaction of the cardiovascular system in the presence of a pathological process in the body by changes in the reactivity of the central nervous system.

In the present research, our aim was to investigate the reactivity of the cardiovascular system to adrenalin and caffeine in experimental noninfectious peritonitis.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits in 4 series (with 5 animals in each). In the first series we studied the character of the reaction of the cardiovascular system of healthy rabbits to the intravenous injection of adrenalin (0.01% solution, 0.2 ml/kg body weight) and in the second series the reaction to injection of caffeine (10% solution, 0.4 ml/kg body weight).

In the third and fourth series of rabbits, 20 hours before injection of the same drugs, peritonitis was induced by injection of 10 ml of a 10% solution of peptone into the peritoneal cavity. The presence of peritonitis was established by an increase of the body temperature, a rise in the leucocyte count and a shift to the left in the leucocyte formula.

During the acute phase of the experiment kymographic tracings were made of the arterial pressure in the common carotid artery. The test substances were injected at the same rate into the femoral vein.

EXPERIMENTAL RESULTS

Analysis of the arterial pressure curves took into consideration the time from the start of injection of the drug until the change in the arterial pressure, the duration of the phase of rise and fall of its maximum and minimum level (Table 1).

TABLE 1

Figures Showing the Trend of the Changes in Arterial Pressure in Response to Injection of Adrenalin (in mm Hg, time in seconds)

	Initial level	Time of beginning of rise	Time of reaching maximum	Maximum level	Duration of phase of falling	Minimum level	Magnitude of rise	Magnitude of fall
				In control animals				
Limits of variation	88—130	3—8	5—18	140—170	43—102	50—100	40—60	60—110
Mean value	105	5	10	155	60	74	50	81
				In experimental animals (with peritonitis)				
Limits of variation	70—95	3—4	4—10	120—160	114—145	20—55	50—70	95—130
Mean value	88	4	7	148	133	37	60	111

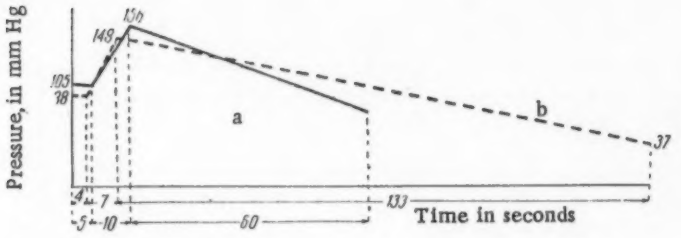

Fig. 1. Trend of the changes in arterial pressure in rabbits after the intravenous injection of adrenalin. a) In healthy animals; b) in those with peritonitis.

Table 1 shows that in response to injection of adrenalin the arterial pressure of both control and experimental rabbits underwent a biphasic change: immediately after injection an increase was observed, and was followed by a fall below the initial level. The same biphasic reaction in response to the injection of adrenalin has been observed by many workers [5, 10, 13, 22]. According to Litwin's [22] findings, the phase of lowering of the arterial pressure is associated with the centrifugal fibers of the vagus nerve, since it almost completely disappears after bilateral vagotomy.

The course of the changes in arterial pressure had its special features in each of our groups of experimental animals (Fig. 1). In the first place the rabbits with peritonitis showed slightly lower initial values. The increase of pressure after injection of adrenalin developed slightly sooner and quicker than in the controls. The maximum level was also slightly lower, although the difference from the initial level was greater in these animals than in the controls.

The time for the pressure to fall from the maximum to the minimum levels in the animals with peritonitis

TABLE 2

Values Showing the Trend of the Changes in Arterial Pressure in Response to Injection of Caffeine (in mm Hg; time in seconds)

	Initial level	Time to reach maximum	Minimum level	Duration of phase of increase	Maximum level	Magnitude of fall	Magnitude of rise
In control animals							
Limits of variation	70—105	2—4	63—96	21—27	81—117	7—13	17—22
Mean value . . .	90	3	80	24	100	10	20
In experimental animals (with peritonitis)							
Limits of variation. . . .	67—100	4—13	40—80	24—24	70—115	20—50	24—60
Mean value . . .	87	8	55	38	97	32	42

was twice as long as in the controls. The pressure in these animals fell more considerably, so that the difference between the maximum and minimum levels was also significantly greater than in the controls.

Hence the cardiovascular system of the rabbits with peritonitis responded more rapidly and intensively to the injection of adrenalin in the first phase (increase of pressure) and more slowly but just as intensively in the second phase (fall of pressure).

When analyzing the changes in the arterial pressure after injection of caffeine, we took into consideration the duration of the phase of fall of pressure to its minimum level, its value and the duration of the phase of rise of pressure and its maximum level (Table 2).

Analysis of these findings showed that the intravenous injection of caffeine was also accompanied by a biphasic reaction of the arterial pressure: at first a fall was observed and then its level rose.

The considerable difference in the trends of the changes in arterial pressure of the control and experimental animals must also be mentioned (Fig. 2). In the latter the phase of fall of pressure was almost three times greater than in the controls. The minimum level of the pressure in these animals was much lower, and the degree of fall three times greater than in the controls. In the rabbits with peritonitis the phase of rise of pressure was more prolonged, but the value of the maximum level was almost the same in the two groups of animals.

The cardiovascular system of the rabbits with peritonitis thus responded more slowly but intensively to injection of caffeine by a fall in pressure in the first phase and responded just as slowly by an increase in pressure in the second phase.

From an analysis of the intensity of the response reaction of the cardiovascular system (Fig. 3), it may be seen that, by comparison with the controls, the arterial pressure in the experimental rabbits with peritonitis was raised either to the same degree after injection of caffeine, or rather more, on the average by 20%, after injection of adrenalin. The fall in pressure, however, was much more sharply expressed in these animals. For instance, after injection of adrenalin the pressure was lowered by 64%, and after injection of caffeine by 220% more than in the controls.

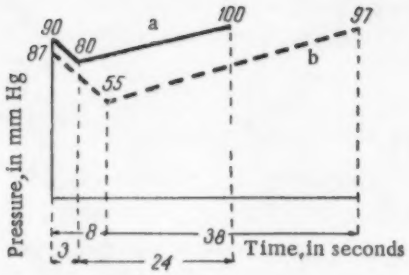

Fig. 2. Trend of the changes in the arterial pressure in rabbits after intravenous injection of caffeine. Legend as in Fig. 1.

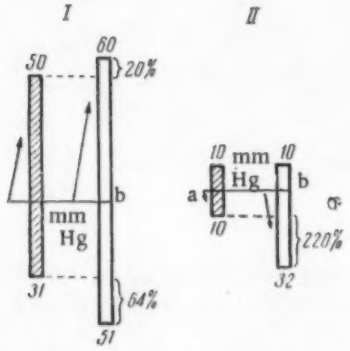

Fig. 3. Magnitude of the rise (upper column) and fall (lower column) of the arterial pressure after injection of adrenalin (I) and caffeine (II) in control (a) and experimental (b) animals.

Thus in the rabbits with peritonitis the depressor reaction was the more marked and prolonged, in response to the intravenous injection of both adrenalin and caffeine.

The reaction of the cardiovascular system in response to the intravascular injection of a chemical stimulus depends on the inclusion of the nervous reflex apparatus of the vascular tissues.

The results obtained show that in peritonitis the reaction of the cardiovascular system to the intravenous injection of these drugs is modified; the degree and duration of the fall in the level of the arterial pressure is much more sharply expressed than in the control animals.

Thus in rabbits with peritonitis, after injection of pressor drugs, the depressor component of the reaction of the cardiovascular system is predominant. At the same time the pressor component shows considerable inertia.

Our results are thus not in agreement with those of D. A. Éskin [21], who found no special features in the changes of the level of the arterial pressure in dogs with peritonitis after injection of adrenalin, nor with the results of G. A. Malov and Ts. I. Vainshtein [7], who showed that in cats with peritonitis the increase in the vascular tone after injection of adrenalin was smaller than in controls, which these authors explained by a lowering of the sensitivity of the veins to adrenalin in peritonitis.

In accounting for the results which we obtained, a whole series of important factors must be taken into consideration. As we know, stimulation of receptors may alter the functional state of the central links of the reflex apparatus and the reactivity of the vasomotor center. For instance, in acute peritonitis changes have been observed in the reactivity of the bulbar centers [19]. In addition it has been shown, morphologically, that in peritonitis a lesion of the autonomic nervous system can be demonstrated [3, 20]. The results obtained, it seems to us, can be explained as follows. The increased flow of afferent impulses in peritonitis raises the level of excitation of both divisions of the autonomic nervous system. The increased tone of the sympathetic system is revealed by a shortening of the latent period and of the time of rise of the pressure to its maximum after injection of adrenalin. The increase in the tone of the parasympathetic system is revealed by the intensive fall in the arterial pressure in the second phase after injection of adrenalin and in the first phase after injection of caffeine. A significant predominance of the depressor reaction over the pressor is observed, however, after injection of both adrenalin and caffeine.

In association with peritonitis a more marked increase in the tone of the cholinergic nerves is therefore observed. This may also be confirmed by the prolongation of the depressor reaction in rabbits with peritonitis. An increase in the sensitivity of both divisions of the autonomic nervous system in acute inflamation was also found by N. V. Puchkov [4].

The experiments of A. G. Bukhtiyarov [1], which showed that depression of the centers leads to predominance of the depressor background, and excitation of the centers to predominance of the pressor background in response to the intravenous injection of drugs, support the possibility of such a mechanism.

SUMMARY

The author studied the reactivity of the cardiovascular system of the rabbit to adrenalin and caffeine in experimental noninfectious peritonitis. On intravenous injection of these drugs against the background

of peritonitis, the cardiovascular system responded by a more intense pressor and depressor effect. The latter, however, was more pronounced, which may point to the higher tone of the cholinergic nerves in peritonitis.

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CHANGES IN THE REACTION OF ANIMALS TO INTRAVENOUS INJECTION OF CAFFEINE AND STROPHANTHIN AFTER RESUSCITATION FROM CLINICAL DEATH

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Restoration of the vital activity of the body after clinical death by means of V. A. Negovskii's [6] method is being increasingly used in Soviet medical practice. The restoration of the action of the heart and of spontaneous respiration are decisive stages in the program of resuscitation of an animal in an agonal state. The appearance of the heart beat and of respiration, however, still does not imply normalization of the basic functions, and still less, restoration of normal health [1, 5, 6, 7].

The work of G. L. Lyuban and his co-workers [2, 3, 4, 5 and others] showed that the reactivity of the animal in the period of restoration of the vital functions after clinical death shows certain essential changes, which are evidently of importance to the effect of both pathogenic and therapeutic agents on the body. Their findings are in agreement with the observations of other authors [6, 8, 9 and others].

It must be mentioned that the reaction of animals which survive clinical death to the administration of certain drugs has not yet been adequately studied. At the same time the investigation of the reaction of the body to different stimuli and in different periods of resuscitation is important in another direction: it assists in explaining the mechanism of action of the etiological factor.

The aim of the present investigations was to study the reaction of animals, which had survived clinical death, to caffeine and strophanthin.

Many factors are known which suggest the importance of the functional state of the body to the character of the action of a particular drug. Drugs such as caffeine and strophanthin may have different effects on the same function, depending not only on the dose of the drug but also on the state of the animal and of its central nervous system. It may be supposed that after the profound anoxia which accompanies clinical death, changes be found in the action of the drugs tested.

EXPERIMENTAL METHOD

Experiments were carried out on 98 cats. Clinical death was induced by exsanguination from the femoral artery, the animals being under light ether anesthesia.

Resuscitation was started after 3-5 minutes of clinical death, by means of intra-arterial injection of blood toward the heart and artifical (mechanical) respiration. Heparin was given preliminarily, and a few ml of physiological saline was added to the blood used for the infusion.

Caffeine or strophanthin were injected intravenously in the first 10 minutes after the appearance of spontaneous respiration.

TABLE 1

Time of Appearance of Convulsions after Injection of Caffeine (6-8 mg/kg of a 10% solution)

Animals	Time after 1st spontaneous inspiration to caffeine inject., in min	Number of animals	Time of appearance of convulsions				
			1 minute	1-5 min	5-10 min	over 10 min	none
Resuscitated	1—4	6	1	1	—	2	2
	4—6	6	1	4	—	1	—
	6—10	8	4	—	2	2	—
All resuscitated		20	6	5	2	5	2
Control		10	9	1	—	—	—

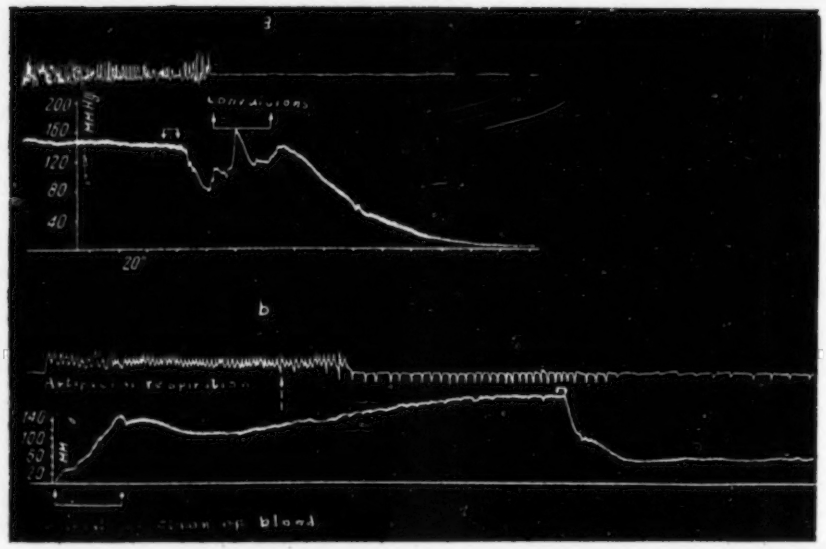

Fig. 1. Reaction to injection of 8 ml/kg of 10% caffeine solution: (a) in a control and (b) a resuscitated animal.

EXPERIMENTAL RESULTS

After injection of a toxic dose — 6-8 ml/kg of a 10% solution of caffeine — to 10 control animals, they all died in the first 10 minutes. Of 20 resuscitated animals, only 3 died at this period; 2 cats died after 10 and 15 minutes; the remainder survived for the 40-60 minutes of observation.

After receiving injections of caffeine the animals developed convulsions, but in the resuscitated animals these appeared later and were less intensive in character.

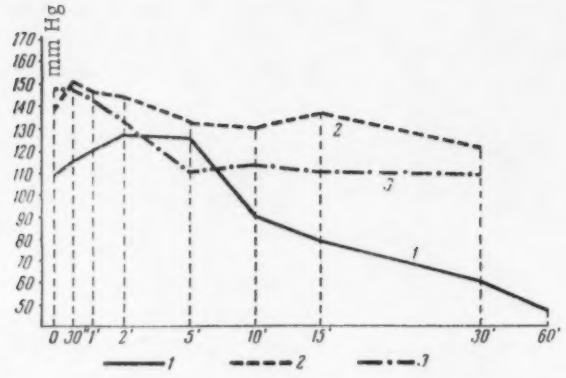

Fig. 2. Changes in the arterial pressure after injection of 1.5 ml/kg of 1% tincture of strophanthin to (1) resuscitated and (2) control animals; resuscitated animals not receiving strophanthin (3).

The longer the animal remained moribund, the longer the period of clinical death and the later spontaneous respiration was restored, the weaker were convulsions.

The time elapsing from the moment of resuscitation was very important: the sooner after the appearance of spontaneous respiration that the caffeine was injected, the less severe were the convulsions (Table 1).

After injection of caffeine the action currents of the heart were severely disturbed. As a rule paroxysmal tachycardia developed and the impulses acquired a polytopic character. In control animals this was followed immediately by a gradual extinction of electrical activity. In the resuscitated animals the heart usually continued working. Cases occurred in which sinus rhythm was restored.

The arterial pressure of the resuscitated animals fell after the injection to 40-50 mm Hg and respiration was temporarily slowed and became more superficial, whereas in control cats respiration ceased at once and the pressure fell to zero (Fig. 1).

In some experiments when, until injection of caffeine into resuscitated animals, their respiration succeeded in acquiring a periodic character, it was found that it returned to an agonal type.

After injection of 1 ml/kg of a 10% solution (larger than the therapeutic dose but not lethal) the difference in the reactions of the resuscitated animals took the form, in the first place, of delay and slight intensity of the convulsions, and secondly, of a considerable reaction of the respiration. After the injection of this dose of caffeine, however, in the resuscitated animals the process of changeover of respiration from an agonal to a periodic type was speeded up slightly.

In control experiments, after injection of 1 ml/kg of 10% caffeine, the arterial pressure fell by 40-50 mm Hg, but rapidly returned to normal, whereas in the resuscitated animals the return to normal was somewhat protracted.

In both the control and the resuscitation animals, after injection of this dose of caffeine a temporary disturbance took place in the work of the heart: the rate was changed, atrioventricular conduction was prolonged the T wave was reduced in amplitude, heart block and paroxysmal tachycardia sometimes developed.

Tincture of strophanthin, like other glucosides, has a selective action on the heart, but has the distinct advantage of rapidity of action and of not causing vasoconstriction. This may be important in the selection of a cardiac glucoside for patients surviving clinical death. In contrast to caffeine, at an early period an increase was observed in the sensitivity to toxic doses of strophanthin (Table 2, Fig. 2).

The more prolonged the course of the agonal state, the clinical death and the resuscitation, the more sensitive the resuscitated animal was to strophanthin. It was interesting to observe that after receiving injections which would be lethal to controls, animals surviving clinical death usually died within a shorter time. There

TABLE 2

Sensitivity of Resuscitated Animals to Tincture of Strophanthin

Dose of 1% solution	Animals	Number of animals	Of which	
			lived	died
1 ml/kg	Resuscitated	10	8	2
	Control	10	10	—
1.5 ml/kg	Resuscitated	10	3	7
	Control	10	8	2
2.5 ml/kg	Resuscitated	9	—	9
	Control	7	—	7

were differences also in the character of the changes in the action currents of the heart, in the respiration and the arterial pressure of the resuscitated animals.

At the moment of injection of 1.0-1.5 ml/kg of tincture of strophanthin, the control animals usually showed an insignificant (within limits of 10-15 mm Hg) and transient fall in the arterial pressure, preceding a subsequent rise. In the resuscitated animals this fall was usually absent, which probably indicated the participation of a reflex component in this reaction.

In contrast to the resuscitated animals, the respiration of the control animals was usually slightly more excited after injection of strophanthin.

After injection of a toxic or lethal dose of strophanthin characteristic changes were observed in the electrocardiogram: the rate and rhythm were disturbed and complete block sometimes developed; the ventricular complex was lengthened; after a certain interval of time ventricular flutter developed. The changes in the electrocardiogram of animals surviving clinical death were usually more pronounced and developed more quickly than in control animals.

These investigations showed that the sensitivity of animals, resuscitated after clinical death, to caffeine and strophanthin was altered. In particular, in the initial period of restoration of the vital functions, a lowering of the sensitivity to caffeine and an increase in that to strophanthin were observed.

A direct relationship was revealed between the changes in sensitivity and the degree of recovery of the function of the body and the degree of involvement of the central nervous system. In this respect, as the convulsive reaction to caffeine showed, even the few minutes from the beginning of recovery of respiration to the injection of the drug are important.

In a previous paper [8] we showed that 30-40 minutes after resuscitation, animals become more sensitive to caffeine than control animals.

The decrease in the sensitivity of the resuscitated animal to large doses of caffeine at the beginning of the restoration of the vital functions may probably be accounted for by the state of severe depression of the central nervous system, and it corresponds to the views of other authors of a diminution of the reaction of the body to toxic substances during profound inhibition of the central nervous system [10].

The enhanced toxic action of strophanthin in the first minutes after resuscitation may be associated with the anoxia to which the animals have been exposed, for this is known to bring about an increase in the sensitivity of the body to cardiac glucosides. A decrease in the gap between the therapeutic and toxic doses of tincture of strophanthin after resuscitation must be taken into consideration when this drug is used.

The results obtained may be important in answering the question of what the prospects may be from the use of caffeine and strophanthin in the treatment of the resuscitated patient, and also in judging the mechanism of action of these drugs. At the same time they add weight to the opinion that the dosage of drugs used in the treatment of patients surviving the terminal state must be reexamined.

SUMMARY

There is a sharp change in the sensitivity to caffeine and strophanthin in cats revived after clinical death by V. A. Negovskii's method. In the first minutes following their revival the sensitivity of cats to caffeine decreases and that to strophanthin increases.

The varied reaction of these substances, differing in their mechanism of action, is consequent upon changed body reactivity after deep anoxia; this should be taken account of in therapy.

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THE MECHANISM OF AMINOPYRINE CONVULSIONS

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Aminopyrine is widely used nowadays in medical practice. Overdosage of this drug may cause many complications such as, for example, dizziness, weakness, convulsions and collapse.

Henkel [3] concluded from his experiments on frogs, in which the central nervous system was transected at different levels, that the convulsive attack which develops after administration of large doses of aminopyrine is depressed by some divisions of the central nervous system and enhanced by others. This author did not, however, discover the mechanism of development of aminopyrine convulsions. It remained uncertain whether these convulsions were the result of a nervous reflex process or whether they developed by automatic stimulation of the central nervous system.

Yu. I. Detsik and I. I. Fedorov [1], when studying the mechanism of aminopyrine convulsions, showed that after intravenous injection of aminopyrine into a subcutaneous leg vein of dogs, without anesthesia, in a dose of 50-60 mg/kg body weight, convulsions quickly developed (at the prick of a needle) with tonic and clonic phases, lasting up to one minute.

When aminopyrine was injected into the suboccipital space, convulsions arose in response to a much smaller dose (8 mg/kg) than after intravenous injection.

On the basis of these findings it may be suggested that the dominant factor in the development of the convulsive attack is the direct action of aminopyrine on the brain substance, but in their later experiments these authors showed that when aminopyrine was injected into the common carotid artery the convulsive attack developed quickly in response to even smaller doses (2.5-3.0 mg/kg body weight) than when it was given into the suboccipital space. They came to the conclusion that the mechanism of the convulsive attack was dependent on the action of aminopyrine on the receptors of the blood vessels, and mainly on the receptors of the carotid sinus.

During perfusion of the isolated carotid sinus of dogs, under morphine anesthesia, with a 4% solution of aminopyrine, I. I. Fedorov and V. I. Zapadnyuk [2] observed slight motor excitation, a quickening of the respiration rate and sometimes whining at the moment of injection, but no convulsions were produced even when the aminopyrine was perfused through both isolated carotid sinuses.

These authors nevertheless assert that the aminopyrine convulsion is reflex in origin. They suggest that for a convulsion to develop it is necessary to have the combined action of aminopyrine not only on the carotid sinus but also on the receptor apparatus of other vascular zones, on the receptors of the internal organs and, possibly, directly on the central nervous system.

The problem of the dominant factor in the mechanism of development of aminopyrine convulsions thus remained unsolved.

It is difficult to agree with these authors' conclusions. In the first place, aminopyrine causes convulsions

when injected into the common carotid artery in much less doses than when injected by other routes. Secondly, during the action of aminopyrine on the isolated carotid sinus no convulsions developed, which is evidence of the direct action of aminopyrine on the brain, which it reached via the internal carotid artery.

In order to elucidate the mechanism of action of aminopyrine we undertook the present investigations.

EXPERIMENTAL METHOD

In all, 29 experiments were performed on 12 dogs.

In the first place we showed that a convulsive attack developed in response to injection of aminopyrine into dogs not only under morphine anesthesia, but also in a state of profound morphine – hexobarbital anesthesia, which we found very suitable for our experiments.

It was also found that under these conditions, the convulsions caused by aminopyrine did not developed in all the dogs (only in 8 of the 12 dogs). Finally, the repeated production of a convulsive attack was, as a rule, impossible within the first two hours of the last attack.

In our experiments the convulsive doses of aminopyrine, injected into the femoral vein or artery, were roughly the same, and amounted to about 30 mg/kg body weight. When aminopyrine was injected into the common carotid artery, convulsions developed with much smaller doses, namely 2.5 mg/kg body weight, which is in agreement with the findings in the literature. Only after the establishement of the convulsive dose by injection of aminopyrine into the common carotid artery were the principal variants of the experiments carried out.

EXPERIMENTAL RESULTS

In the first series of experiments we studied the effect of aminopyrine when injected into the common carotid artery after preliminary ligation of the internal carotid artery. As has already been pointed out, on injection of aminopyrine into the common carotid artery without preliminary ligation of the internal carotid artery, convulsions developed in response to a dose of 2.5 mg/kg body weight.

After preliminary ligation of the internal carotid artery, no convulsions developed in these same dogs even after injection of much larger doses of aminopyrine into the common carotid artery (5 mg/kg and 12.5 mg/kg and over), in spite of the possibility of the drug acting on the receptor apparatus of the carotid artery. In these conditions a convulsive attack could be produced, however, if aminopyrine was injected in doses exceeding the intravenous doses, i.e., 30 mg/kg. In the dog Polkan, for instance, convulsions developed only when the dose of aminopyrine was increased to 50 mg/kg body weight.

On the basis of these experiments it may be postulated that the essential link in the mechanism of production of convulsions is the automatic action of aminopyrine directly on the brain substance. This view is also supported by the results obtained in the next series of experiments, in which the external carotid artery was ligated and the aminopyrine was injected into the common carotid artery. In these experiments the aminopyrine passed through the internal carotid artery directly to the vascular system of the brain and convulsions developed rapidly (to the prick of a needle) with a dose of 2.4–2.0 mg/kg body weight.

In this particular case the possibility of reflex action from the carotid sinus reflexogenic zone is not excluded. In this connection it might be thought that important factors in the mechanism of the convulsive attack are both the direct action of aminopyrine on the brain and its reflex action via the carotid sinus reflexogenic zone.

For this reason, in some experiments, after ligation of the internal carotid artery the aminopyrine was injected into that vessel peripherally to the point of ligation, thereby avoiding the carotid sinus reflexogenic zone. It was found that to obtain a convulsive attack under these conditions required a much smaller dose (1.0–0.6 mg/kg body weight), a dose which when injected, failed to produce a convulsive attack in any other experiment which we carried out.

On the basis of the results obtained, we conclude that the main link in the mechanism of production of aminopyrine convulsions is the direct action of the drug on the brain, and not its reflex effect from the various vascular reflexogenic zones.

SUMMARY

When injected into the common carotid artery the convulsive dose of aminopyrine in dogs was 2.5 mg/kg, while into the femoral artery or vein it was about 30 mg/kg. After unilateral ligation of the internal carotid artery the convulsive dose of aminopyrine injected into the ipsilateral common carotid artery increased greatly and even exceeded the intravenous dose. Following unilateral ligation of the external carotid artery a dose of less than 2 mg/kg of aminopyrine induced convulsions, when injected into the common carotid artery of the same side. The convulsive dose of aminopyrine was even lower (0.6 mg/kg) when injected directly into the internal carotid artery (following ligation of this vessel below the site of injection).

Hence, the direct effect of this drug on the brain is to be regarded as the principal link in the mechanism of convulsions induced with aminopyrine.

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ONCOLOGY

THE INHALATIONAL METHOD OF TRANSPLANTATION OF TUMORS

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The problem of lung cancer in man has recently been widely discussed in the oncological literature. The interest in this problem is due to the increase in the number of cases of this disease among the population in various countries, especially in large cities and industrial centers [4, 6, 7]. In connection with the increasing attention which is being paid to lung cancar in man, several questions arise, which call for experimental investigation.

It is known that patients with cancer of the lungs may excrete into the external environment isolated tumor cells or groups of cells, which have become detached from tissues that are being destroyed [1, 2]. Is it possible for "infection" (homotransplantation) of these tumor cells to take place in persons near the patient?

N. N. Petrov [5], describing some unsuccessful experiments on inoculating human subjects with tumors from other patients, pointed out that "it would be quite untrue to conclude from these solitary facts that inoculation of human tumors cannot succeed in man" (p. 270). In numerous experiments carried out at the beginning of the experimental era of oncology, transplantations were usually done in animals whose origin was unknown and which had different hereditary features. In some cases, few it is true, these experiments were successful, especially if the transplantations were made from parents to offspring, if the recipients were young and the tumor possessed powers of malignant, infiltrative and metastatic growth (N. N. Petrov, 1947). Is it therefore possible to completely exclude "infection" (homotransplantation) of members of a family, especially the children of a patient, by inhalation of lung cancer cells which have been excreted into the external environment? Is the inhalational transplantation of tumors generally possible? These problems formed the background to our experiments on the study of the inhalational method of transplantation.

There are indications in the literature of the successful transplantation of tumors by the inhalational route [3, 9, 10], but the tumors studied in these investigations were transplanted tumors. M. M. Maevskii and T. M. Maevskaya [3], for example, used Ehrlich's carcinoma — an old laboratory strain of the tumor — repeatedly transplanted intraperitoneally in mice, and Schmidt [9, 10] used several laboratory strains of tumors. The relative ease of transplantation of these strains in the lung may be due to the adaptation of these tumors to animals in the course of prolonged transplantation.

We decided to test the possibility of inhalational transplantation of a spontaneous tumor located in the lungs. So far as we know, such an investigation has never been made before. Nevertheless it would most closely reproduce the conditions appertaining to cancer of the lung in man. By using material from a recent case of malignant disease, and not a laboratory strain of tumor which had undergone prolonged passage, we might, to some extent, obtain the answer to our question. By chance we were able to select some suitable material.

EXPERIMENTAL METHOD

A spontaneous tumor of the mammary gland was found in female mouse No. 675, belonging to the high-cancer C_3HA line, in the 12th month of life. At autopsy it was found that, besides the tumor of the mammary gland, the mouse had multiple tumor nodules in both lungs (Fig. 1).

Histological examination showed that the mammary gland tumor was an adenocarcinoma and that the tumors in the lungs were metastases of this adenocarcinoma. This was thus a tumor, arising in natural conditions and having, as a result of metastasization, the situation which we needed — in the lungs.

A finely minced suspension of tissue in physiological saline was made from the nodules of this tumor, and injected intranasally into 4 10-week-old mice of the same line C_3HA in which the tumor had developed. Inhalation was carried out under ether anesthesia. The dose of tumor suspension administered was 0.03-0.05 ml.

EXPERIMENTAL RESULTS

One of the experimental mice died from an accidental cause 2 weeks after transplantation; no tumor was found in this animal's lung. In 3 mice, 10 weeks after transplantation, we found quickened breathing, and 11 weeks from the beginning of the experiment they were sacrificed. In all 3 animals well-developed tumors were seen in the lungs, and in two of them more than half the lung tissue was replaced by tumor. Histologically these tumors duplicated the structure of the original tumor. The lung tumor of one of these mice was transplanted by inhalation to 4 fresh mice of the C_3HA line. Two weeks later, one mouse developed a tumor of the lung. Futher transplantations of this tumor led to the development of similar tumors in the third and fourth generations.

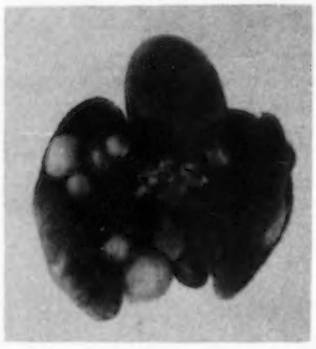

Fig. 1. Metastases of a spontaneous adenocarcinoma of the mammary gland in the lung of female mouse No. 675.

The experiments thus gave a convincing answer to the question which had been asked on the possibility of homotransplantation of cells of a spontaneous tumor by the inhalational method. In these experiments, however, the conditions, for such a transplantation had been the most favorable possible: a relatively large dose of tumor suspension was inhaled directly into the respiratory tract of the animals.

The question naturally arose whether it was possible for tumors to developed in the lung by inhalation of tumor cells in ordinary conditions. On a priori grounds it might be thought that the probability of such "infection" was infinitesimally small. Nevertheless we carried out an appropriate experiment. Young mice of the same pure line were reared together with mice affected by tumors of the lungs. Observations were made on both the newly introduced young mice and on the offspring of these mice and those with the tumors. We considered the observations on the offspring to be interesting in view of the fact that young, and especially newborn mice are more susceptible to homotransplantation [8, 9, 10]. In the course of 2 years of observation we found no case of "contact infection": no tumor was found in the lungs of a single newly introduced mouse or of the offspring of the experimental mice. It must be remembered that in our experiments the mice with tumors of the lungs and the mice in contact with them belonged to the same inbred line.

Thus although our experiments showed that by inhalation of a suspension of cells from a spontaneous tumor, homotransplantation of the inhaled tumor cells is possible and tumors may develop, situated in the lungs, the chances of success in homotransplantation of this sort in normal conditions are infinitesimally small.

In natural conditions, patients with malignant neoplasms of the lungs excrete into the external environment cells detached from disintegrating tumors. Under these circumstances it is not impossible for a viral agent to be liberated and spread. The extensive clinical material, however, points to the absence of cases of contact infection of cancer of the lung. It is probable that this path of spread of the carcinogenic agent is of no practical importance although, of course, much of the material must be reexamined in the light of modern knowledge of the greater susceptibility of young animals (especially newborn) to cancer-producing virus and of the long latent period of these tumors.

During transplantstion of pulmonary metastases of spontaneous adenocarcinoma of the mammary gland in a mouse into the lung, we noted a number of peculiarities of the growth of these tumors. They increased in size much more slowly than did the same tumors transplanted subcutaneously. In the case of primary transplantation, for instance, when subcutaneous transplantation was carried out at the same time as transplantation into the lung, large tumors, suitable for transplantation, developed in 3 weeks, whereas such tumors developed in the lungs in $2\frac{1}{2}$ months. In the subcutaneous tumors areas of extensive necrosis were observed, but these were few in the lung tumors. In mice sacrificed in the initial stage of growth of the tumor (on the 20th-25th day after transplantation), there were small, greyish nodules of tumor tissue, the size of a pin head, situated in the lung, in areas of lung tissue which were macroscopically unchanged. In mice dying from lung tumors, or sacrificed when signs of tumor were present – severe dyspnea and wasting ($2-2\frac{1}{2}$ months after inhalation) – in the lungs there were either

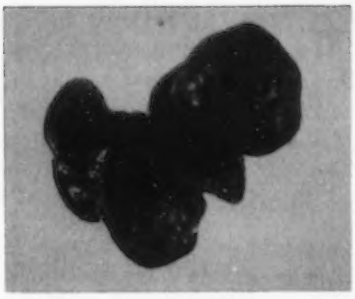

Fig. 2. Tumor of the lung developing after intranasal inhalation of a suspension of adenocarcinoma of the mmary gland. Large tumor nodules, almost completely replacing lung tissue.

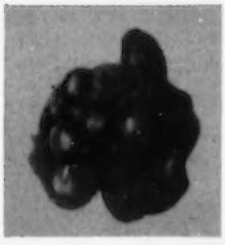

Fig. 3. Hepatoma – a tumor in the lung, developing as the result of inhalational transplantation.

solitary, large tumor nodules, occupying almost the whole of the thoracic cavity, reaching a diameter of 1.2 cm, compressing the heart and the residual lung tissue (Fig. 2), or a large number of smaller nodules, hanging in bunches from the hilum of the lung, almost completely replacing the lung tissue and also compressing the thoracic organs. The weight of the tumors, in such cases, filling the thoracic cavity, reached 1.5 g. The survival period of the animals with a tumor developing after inhalation of a suspension of a spontaneous tumor was longer than in cases where the same tumor was transplanted subcutaneously into the same mouse.

In order to ascertain whether these distinctive features depended on the transplantation of this particular spontaneous tumor of the mammary gland, we tried the method of intranasal transplantation also with certain transplanted strains of mouse tumors.

We had available a hepatoma, transplanted in mice of line C_3HA, and 2 adenocarcinomas: one derived from a spontaneous tumor of a C_3HA mouse and transplanted subcutaneously into mice of the same line, and the other derived from a spontaneous adenocarcinoma in a C57 mouse (black) and transplanted subcutaneously into mice of line C57 (black). As might have been expected, in accordance with data in the literature [3, 9, 10], all 3 tumors were readily transplanted by intranasal infection, and gave rise to growth of tumors in the lungs of the mice (Fig. 3). The transplanted tumors retained the histological structure of the original tumors.

It must be mentioned that success in intranasal infection depends to a large extent on the correct anesthesia of the animal and throughness of the infection. In deep sleep, mice readily inhale small droplets of the suspension which enables tumor cells to pass through the trachea and bronchi into the lung tissue. If the anesthesia is light or the suspension is inhaled in large drops, inhalation of the liquid is interfered with, it is scattered by the expired air and the success of the transplantation is jeopardized. These findings are in agreement with the observations of M. M. Maevskii and T. M. Maevskaya [3].

Transplanted strains of tumors, when inoculated intranasally, possess the same properties as were noted during transplantation of a spontaneous tumor into the lung: slow growth, relatively feeble development of necrotic processes, longer survival of the animals affected by the tumors. This makes the method of intranasal inoculation of animals particularly suitable for the study of certain problems of the immunology of tumors which require comparatively long periods of observation, such as for example, the increase in antibody titers during the growth of tumors. Slowly developing tumors in the lungs may be used as models for the study of the problem of vaccination and for investigating in vivo the action of specific antitumor antibodies.

The intranasal method of transplantation of tumors may be used for the heteroptransplantation of tumors in general and, in particular, of cancer of the lung in man. Finally, the tumor, growing in the lung, is a suitable model for the study of the action of chemoterapeutic drugs when given by the inhalational method.

SUMMARY

The inhalation of a cell suspension of a spontaneous tumor (metastases in the lung of spontaneous adenocarcinoma of the mammary gland in C_3HA mice) results in homotransplantation of the inhaled cells with the development of tumors in the lung. Observations of natural contacts of healthy mice with animals having transplanted pulmonary tumors for a period of 2 years failed to reveal such transplantation.

The inhalation method of tumor transplantation may also emplored for inoculating transplantable laboratory strains, as well as for transplantation of naturally occuring tumors in mice. The inhalation method is of help in maintaining the tumor strain localized in the lung during passage.

Tumors developing in the lungs as the result of inhalation transplantation have some peculiarities: a longer duration of growth in comparison with subcutaneous inoculation, slow development of necrotic processes, and longer survival of the mice suffering from this tumor. All this makes this method convenient for solving a number of experimental oncological problems.

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COMPARISON OF THE ESTROUS CYCLES IN MICE OF HIGH AND LOW CANCER LINES

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Many experimental investigations and clinical observations have now shown that the development of the different forms of pathological proliferation of the epithelium of the mammary glands, the so-called mastopathies and carcinoma of the mammary glands into which they often are transformed, are associated with disturbance of the balance of the estrogenic hormones and the hormones of the adenohypophysis in the animal body. Since the anterior lobe of the hypophysis secretes several hormones which act on both the ovaries and the mammary glands, and the ovary, in turn, acts on the hypophysis with its own hormones, the hormonal interralationships between these organs are highly complex.

Oncologists and endrocrinologists have carried out numerous experimental investigations dealing with the hormonal balance both in mice often affected by carcinoma of the mammary glands and in mice resistant to carcinoma. Attempts to discover the nature of the hormonal disturbances in the so-called high cancer lines of mice, by comparison with the low cancer lines, have been far from successful.

One of the experimental methods enabling the hormonal function of the ovaries and the hypophysis to be assessed in the method of study of the estrous cycle. The character of the estrous cycle in individual animals reflects the inidividual balance between the ovarian and hypophyseal hormones and the rhythm of secretion of these hormones.

The duration of the various stages of the estrous cycle in mice of high and low cancer lines has been studied by many workers, but their findings are extremely contradictory.

Some workers [6, 7] assert that in mice of high cancer lines estrus is much more prolonged and diestrus much shorter than the corresponding stages of the estrous cycle in mice of low cancer lines. According to the findings of other workers [1, 3, 5], no differences can be found in the total duration of the estrous cycle and in the duration of its various stages in mice of low and high cancer lines.

The aim of our investigation was to compare the characteristics of the estrous cycles in mice of high and low cancer lines, and also to study the influence of the time of year on the course of the estrous cycles.

EXPERIMENTAL METHOD

Experiments were carried out on 40 female mice of known lines. As a high cancer line we selected line "A", and as low cancer line "C_{57}" (black). Vaginal smears were taken twice a day from the mice for a period of 3 weeks, stained by the Romanowsky — Giemsa method and examined under the microscope. Mice in which no cyclic changes were found in the vagina were excluded from the experiment. In order to detect seasonal changes in the estrous cycles, in a first series of experiments vaginal smears were investigated in February, and in a second series, in May.

Estrous Cycles in Mice of Lines "A" and "C_{57}"

Time of year	Line	Number of animals	Duration of experiment, in days	Mean duration in days, of whole cycle	estrus	metestrus	diestrus	proestrus
February	«A»	10	21	5,19	2,28	0,80	1,68	0,43
February	«C_{57}»	11	22	5,38	1,48	0,54	2,95	0,42
May	«A»	9	22	5,80	2,09	0,76	2,36	0,60
May	«C_{57}»	10	22	5,80	1,52	0,71	2,97	0,58

EXPERIMENTAL RESULTS

The investigations showed that in healthy mice, kept a normal room temperature during the winter months, the estrous cycles did not cease. In each of the mice investigated, the estrous cycle was individual in its character. Furthermore, in the majority of mice, the duration of the individual stages of the estrous cycle changed in the course of the experiment. It was impossible to detect any relationship whatever between the character of the estrous cycle and the age of the mice.

The character of the winter and spring cycles in the mice is illustrated in the table.

A characteristic feature of some animals of line "A" was the sudden onset of an extremely prolonged estrus, which was followed by a relatively correct alternation of the stages in their usual length. The duration of estrus in these cases reached 4-8 days, but diestrus, on the other hand, was considerably shortened. In the majority of line "A" animals the length of diestrus in the individual cycles was shortened to 1-1$\frac{1}{2}$ days. In the animals of the low cancer line, in no case did estrus last longer than 2$\frac{1}{2}$ days, and in occasional cycles it was shortened to 24 hours. In many of the females of this line diestrus was extremely prolonged, lasting for 5-13 days. When the mean values were calculated (see table), the extremely individual differences which were characteristic of each line were to a large extent concealed.

Comparison of the estrous cycles of lines "A" and "C_{57}" in February shows that the mean duration of estrus was much longer in mice of line "A" than in those of line "C_{57}". So far as diestrus was concerned, here the opposite relationship was observed, i.e., in the mice of line "A" the duration of diestrus was much less than in the mice of line "C_{57}". Statistical analysis of the mean values of the duration of estrus and diestrus in the mice of these lines showed that the differences described above were significant (P=0.0001). The mean duration of the estrous cycles in these two lines was almost the same. It also follows from the figures in the table that metestrus in mice of line "C_{57}" in winter was much shorter than in mice of line "A".

From a comparison of the winter and spring cycles it can be seen that the duration of estrus and diestrus in the mice of line "C_{57}" hardly changed in the course of the year. The total length of the cycle increased slightly in May, and there was a corresponding increase in the duration of proestrus and especially of metestrus. The physiological meaning of these phenomena is probably an increase in the possibility of fertilization, since fertilization in mice occurs at the end of estrus and during metestrus [4].

In contrast to the "C_{57}" line, in the females of line "A" the spring pattern of the estrous cycle was changed (see table). In the first place attention was drawn to the considerable lengthening of diestrus, which did not, however, reach the mean length of diestrus in the mice of the low cancer line. Conversely there was a slight decrease in the duration of estrus. The whole cycle was lengthened, just as in the case of the mice of line "C_{57}". The duration of metestrus and proestrus was essentially unchanged.

Comparison of the cycles in the mice of lines "A" and "C_{57}" in May showed that the relationship which had been observed when these cycles were compared in February was maintained, although it was more feebly expressed. As before, estrus was more prolonged in the mice of line "A", and diestrus was shortened by comparison with the duration of these phases in the line "C_{57}" mice. Statistical analysis showed that the difference between these values was marginally significant (P = 0.03). Thus in spring, in the period of high sexual activity and intensive reproduction, the character of the sexual cycle of the mice of the high cancer line approximated to the character of the sexual cycle of normal mice.

Our findings were in agreement with the experimental results of Lacassagne [7] and Harde [6], and in disagreement with the conclusions of Bonser [5], L. P. Grigoliya [1] and N. I. Nuzhdin [3].

The cause of this discrepancy between the results is very difficult to account for. Estrous cycles in different high cancer lines and sublines of mice may possibly have essential differences and, although Lacassagne's, Harde's and our own results were obtained in mice of different lines, it is impossible to transfer them to all high cancer lines of mice. In this way it is easy to explain the difference in Bonser's results. It is far more difficult to find the reason for the discrpancy between our findings and the experimental results of N. I. Nuzhdin and his co-workers [3], since we obtained our families of high cancer line mice from the same nursery. Possibly in this case the character of the cycles was affected by differences in the conditions under which the mice were kept. In the Institute of Genetics of the AN SSSR the mice were kept in an artificially illuminated basement, and in our institute in a room in sunlight. Other differences were present in their conditions of keeping.

The method of conduct of the experiments differed considerably. We took smears from the mice not once but twice a day, and our experiments were much shorter in duration. It seems to us that our method was superior for when smears are taken only once a day it is easy to miss the short stages of the estrous cycle (proestrus and metestrus) and erroneously to combine two cycles into one, and in any case it is impossible to determine accurately the lengths of the individual stages of the cycle. The prolonged taking of vaginal smears often leads to distortion of the estrous cycle and even to the development of a state of pseudopregnacy.

We received the impression that the estrous cycles were extremely labile and easily altered their character under the influence of many exogenous and endogenous causes. In spite of the negative results obtained by several authors, the question of the peculiarities of the estrous cycles in mice of high cancer lines cannot, therefore, be regarded as answered. The conclusive solution of this problem is of great interest to experimental oncology. If the estrous cycle in high cancer lines of mice really presents special features, it will be possible to use these features to define the estrous cycle or, in other words, the hormonal type, characteristic of animals developing carcinoma of the mammary gland soonest. By using the type of estrous cycle as a prognostic sign, it will be possible not only to select animals for an experiment, but also to maintain high cancer lines of animals without having to cross only brothers and sisters, which give grossly weakened generations.

High cancer mice are known to be less fertile than ordinary mice of no pure line. It is very probable that this is determined by peculiarities of their hormonal balance. It is all the more strange that, in breeding high cancer lines of mice in many institutions, the ordinary method of selection is used: selection for breeding of the most fertile females. In this case, however, high fertility is a sign of a normal hormonal balance. Is this not the reason why many high cancer lines of mice gradually lose their specific characteristics, and why the incidence of development of tumors among them falls considerably [2].

SUMMARY

In February and May the author studied the duration of the estrous cycle stages in female mice of high "A" and low "C_{57}" cancer lines. It was established that the average duration of the estrus is much greater and of diestrus much shorter in the "A" than that of the corresponding stages in the "C_{57}" line.

The estrous cycles of the "C_{57}" line females do not undergo any significant seasonal changes while in the "A" line they approach the character of the normal mice spring cycles.

Desviations from the normal duration of the estrous cycle stages in the high cancer line mice denote a disturbed hypophyseal - ovarian hormonal balance.

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CHANGES IN THE ONCOLYTIC PROPERTIES OF THE SERUM OF RATS WITH GUÉRIN'S CARCINOMA UNDERGOING TREATMENT WITH SARCOLYSIN

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The use of the oncolytic reaction for assessing the effectiveness of the various methods of treatment of tumors has been investigated, from reports in the literature, only to a very slight degree. Only from isolated pieces of research has data been obtained on the oncolytic activity of the sera of cancer patients before and after surgical removal of the tumors. There are, thus , indications that after the radical surgical treatment of cancer patients the oncolytic activity of the serum increased. In cases where a nonradical operation was performed the oncolytic activity remained at a low level, and as the clinical course of the disease became aggravated, it fell still further [2, 4-7]. The changes in the oncolytic activity of the sera of animals and man with malignant disease treated by chemotherapy, so far as we can judge from the accessible literature, has not been studied at all.

Nowadays, when chemotherapy is beginning to occupy an important place in the treatment of certain forms of malignant disease, the search for objective methods of evaluation of the efficacy of drugs is a task of some urgency. From observations of an increase in the oncolytic properties of the sera of cancer patients after their successful surgical treatment, it may be thought that the reaction of oncolysis might serve as one such method.

In this paper we describe the results of one of our experiments directed toward the verification of this hypothesis. In the research we used a method of determination of the oncolytic properties of serum which we ourselves suggested, based on the principle of photometry [1].

EXPERIMENTAL METHOD

Fourteen rats with a positive oncological serum index i.e., their sera possessed the property of lysing tumor cells) were inoculated subcutaneously with a suspension of cells of a Guérin's rat carcinoma in a dose of 0.5 ml. On the 7th day after inoculation of the tumors, when they had reached measurable dimensions (mean diameter 6.5 mm*), we began to treat all the rats of this group with sarcolysin in a dose of 5 mg of the drug per kg body weight. Altogether 7 intraperitoneal injections of sarcolysin were given to each animal at 72-hour intervals. In the course of sarcolysin treatment the oncolytic activity of the serum was measured periodically.

* We measured the tumor with calipers in 3 directions: length (along the length of the rat's body), width (across the rat's body) and height of the tumor, in mm; we then calculated it mean diameter from the formula 3 abc, where a, b and c are the length with and height of the tumor respectively.

TABLE 1

Oncolytic Indices of the Sera of Rats with Guérin's Carcinoma Treated with Sarcolysin, and of Control Rats.

Serial No.	Rat No.	Oncolytic index before tumor inoculation	after inoculation of tumor: 6th day	9th day	21st day	27th day
Rats with Guérin's carcinoma, treated with sarcolysin						
1	55	+15	−18	16	+14	+ 4
2	65	+13	−16	−10	—	+ 9
3	66	+49	−20	−20	+ 5	+11
4	46	+12	−15	− 9	+ 4	+ 3
5	48	+ 7	− 2	0	+ 7	+10
6	64	+ 7	− 7	− 3	− 1	− 4
7	50	+ 6	−12	−12	+10	+ 3
8	42	+11	−14	−11	+10	+ 6
9	45	+35	−16	− 9	− 8	+ 4
10	67	+37	−14	−14	+10	+27
11	68	+77	−17	−17	+ 3	—
12	51	+12	−14	−16	+10	+ 8
13	71	+28	−16	−14	+11	+13
14	29	+ 6	−17	−10	+ 6	+ 6
Healthy rats receiving sarcolysin						
1	37	+11	+10	−16	+12	+ 2
2	49	+39	+39	− 5	+ 7	+ 5
3	58	+ 4	+ 6	−15	+15	+32
4	47	+ 6	0	−16	+12	+ 5
5	33	+15	+ 5	−14	+ 9	+ 2
6	34	+21	+18	−13	+ 5	+ 2
7	27	+ 3	+ 4	−12	+18	+23
8	26	+30	+31	+31	+19	+20
9	30	+ 7	+ 7	−17	+14	+ 7
10	70	+ 5	+ 4	−18	—	− 3
11	56	+ 7	+ 8	−12	+14	+ 6
12	69	+ 6	+10	−17	—	—
13	72	+41	+40	−19	+ 8	+31
Rats with Guérin's carcinoma, not receiving sarcolysin						
1	36	+14	−17	−16	0	0
2	23	+15	0	+ 1	0	−16
3	25*	+28	+ 6	+13	0	Died
4	16	+ 8	−10	− 9	− 7	− 9
5	39	+41	−18	−17	−17	−20
6	13	+36	−15	−12	−10	−11
7	18	+73	−10	− 6	−11	−15
8	62*	+11	−16	−17	−11	Died
9	31	+ 6	−11	−10	− 9	−19
10	24*	+ 7	− 9	−10	− 7	Died
11	21	+35	+10	+18	+ 5	−12
12	17	+ 5	− 9	− 7	−20	−29
13	22	+ 8	−20	− 8	− 8	−15
14	38	+ 6	−20	−19	−20	−21

* Rats Nos. 25, 62 and 24 died from the tumor.

As controls we took 2 further groups of rats (13 animals in one group and 14 in the other). The oncolytic index of the sera of all the rats included in the two positive. The animals of one of these groups were inoculated subcutaneously with a suspension of cells of a Guérin's rat carcinoma at the same time as the rats of the experimental group and also in a dose of 0.5 ml. The second control group consisted of rats which received no tumor inoculations, but in which the injections of sarcolysin and measurements of the oncolytic activity of the sera were carried out at the same times as in the experimental groups.

EXPERIMENTAL RESULTS

The experimental results obtained are shown in Tables 1 and 2 and the figure.

As can be seen from Table 1, in all the rats of the first group, before inoculation of the tumor, the sera possessed well-marked oncolytic activity (shown by positive figures). On the 6th day after inoculation of the tumor the oncolytic index (OI) was in all cases negative, which indicated a sharp fall in the oncolytic activity of the sera.

On the 9th day after inoculation of the tumor, depression of oncolytic activity was observed as before; the OI in all cases was negative. On the 21st day the OI of the serum of all the animals except two (rats Nos. 45 and 64) once more became positive, which was evidence of an increase in the oncolytic activity of these rats. In 4 rats (Nos. 29, 42, 48 and 55) the oncolytic activity was completely restored to its initial (before inoculation of the tumor) level. In only the two rats (Nos. 45 and 64) did the OI continue to fall. On the 27th day a further increase in the OI of the sera was observed in 5 animals (Nos. 45, 48, 66 and 71); in another 5 rats (Nos. 42, 50, 51, 55 and 65) the OI, on the other hand, fell slightly, and in rats Nos. 29 and 46 it remained at its former level. So far as the control animals were concerned, whose sera also possessed well-marked oncolytic activity, in the group of rats in which the tumor was not exposed to the action of sarcolysin a sharp fall in the oncolytic activity was observed on the 6th day after inoculation of the tumor, and this had not recovered by the time the experiment ended. In the group of animals which were not inoculated with the tumor but which received sarcolysin, on the 6th day the oncolytic activity of the serum was unchanged in the majority of the rats, and only in one (rat No. 47) was it decreased. On the 9th day the OI of the serum in all the rats except one (rat No. 26) fell sharply. On the 21st day the serum of all the animals tested had again acquired the property of lysing tumor cells. On the 27th day, by comparison with the previous days of

TABLE 2

Mean Diameters of Tumors in Rats with Guérin's Carcinoma and Treated with Sarcolysin, and in Control Rats

Serial No.	Rat No.	Mean diameters of tumor at different times after inoculation (during sarcolysin treatment)		
		7th day	14th day	26th day
		in mm		
Rats with Guérin's carcinoma and treated with sarcolysin				
1	55	9,1	11,0	7,4
2	65	9,5	10,2	12,1
3	66	9,4	13,0	7,1
4	46	11,0	13,2	9,7
5	48	10,1	8,4	4,2
6	64	9,9	6,8	3,6
7	50	8,5	7,4	7,2
8	42	4,8	6,8	6,5
9	45	6,8	6,8	4,9
10	68	9,2	18,1	7,3
11	68	4,5	7,5	4,2
12	51	8,9	9,9	7,5
13	71	8,2	10,1	8,7
14	29	8,9	6,5	0
Rats with Guérin's carcinoma and not treated with sarcolysin				
1	36	5,0	21,5	30,2
2	23	9,1	23,2	31,0
3	25*	7,8	23,0	Died
4	16	7,3	21,0	34,2
5	39	5,4	20,2	45,0
6	13	4,8	10,0	43,0
7	18	5,3	17,4	39,0
8	62*	7,7	24,4	Died
9	31	9,4	29,3	48,0
10	24*	6,9	20,0	Died
11	21	9,3	24,0	34,0
12	17	7,9	22,4	44,0
13	22	6,5	21,8	45,0
14	38	6,2	21,8	36,2

* Rats Nos. 25, 62 and 24 died from the tumor.

the investigation, some rats showed a slight fall in the OI ; in others on the other hand, the value of the OI rose slightly, apart from in rat No. 70 in which the value of the OI at this time was −3. In other words, on the 27th day the serum of all the rats except one (rat No. 70) had once again acquired the property of lysing tumor cells.

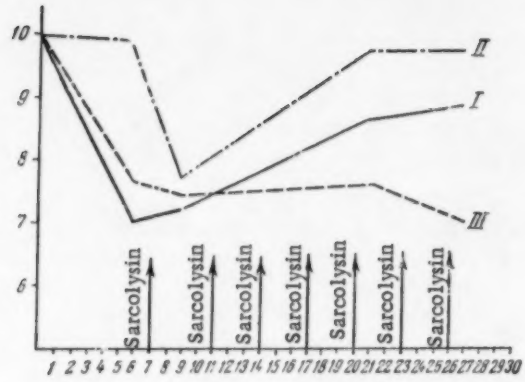
The results of the experiment thus showed that in animals with tumors and treated with sarcolysin, on the 21st day an increase was observed in the oncolytic activity of their sera, which persisted until the animals' death (as the result of the development of an epizootic). In the animals with tumors and not treated with sarcolysin a steady loss of the oncolytic properties of the serum was observed. The oncolytic activity of the sera of normal rats under the influence of sarcolysin at first fell. This fall was, however, of short duration, since on the 21st day the sera of all the animals had regained the property of lysing tumor cells.

In Table 2 are shown the results of measurement of the tumors in rats belonging to the first and third groups. We see that on the 26th day the tumor developing in one of the rats treated with sarcolysin (rat No. 29) had completely absorbed. The tumors in the remaining 9 animals, although they had not undergone absorption, had nevertheless begun to regress; in one rat the dimensions of the tumor were unchanged on the 26th day, and only in one (rat No. 65) had the tumor at this time slightly increased in size. In the group of animals with tumors and not treated with sarcolysin, the tumors in all the rats grew rapidly and continuously As a result of growth of the tumors, 3 rats died on the 26th day.

From a comparison of the results shown in Tables 1 and 2 it will be seen that the increase in the oncolytic power of the sera of the treated animals on the 21st and 27th days was accompanied by a decrease in the size of the tumors on the 27th day . Conversely, the continuous fall in the oncolytic activity of the sera in the group of animals not treated with sarcolysin was accompanied by a steady rise in the dimensions of their tumors. A certain increase in the oncolytic activity of rats Nos. 24, 25 and 62 on the 27th day may evidently be explained by the fact that the oncolytic index of animals is raised before death, as was shown by V. I. Sachkov [3].

For greater clarity, the experimental results are depicted in the form of curves on the figure. Along the ordinate axis are plotted the indices of oncolytic activity of the sera (coefficient of definition 10), and along the abscissa the time elapsing after inoculation of the tumor (in days). Curve I shows a sharp fall on the 6th day. After the 9th day it rises to reach its maximum height on the 27th day. This peak of the curve corresponds to the period when the tumors were reduced in size or were completely absorbed. This clearly suggests that sarcolysin had a definite effect on the animals of this group, inhibiting growth of the tumor and favoring the restoration of the oncolytic power of the sera of these animals.

As may be seen from curve II, the administration of sarcolysin to the animal itself caused a fall in the oncolytic properties of the serum; this fall, however, was only temporary. On the 18th-24th day the animals became habituated to sarcolysin and the oncolytic activity of their sera again rose. The fall in the oncolytic activity of the sera under the influence of sarcolysin must be taken into consideration when analyzing the changes



Trend of the changes in the oncolytic activity of the sera of rats with Guerin's carcinoma and treated with sarcolysin. I) Changes in the oncolytic activity of the sera of rats with tumors under the influence of sarcolysin; II) changes in the oncolytic activity of the sera of normal rats under the influence of sarcolysin; III) changes in the oncolytic activity of the sera of rats with tumors and not treated with sarcolysin.

in this activity in animals with tumors, treated with sarcolysin. Presumably if sarcolysin did not have the action of lowering the intensity of oncolysis, curve I would have been higher (the oncolytic activity of the corresponding sera would have been higher). Curve III illustrated the continuous fall in the oncolytic activity of the sera of animals with tumors, not treated in any way.

It can thus be concluded that if sarcolysin treatment has an inhibitory action on the growth of the tumor, this is quickly reflected in the state of the oncolytic power of the sera of the treated animals: an increase in their oncolytic activity is observed; conversely, as the malignant disease progresses, the oncolytic properties of the serum rapidly diminish.

In our view the results obtained suggest that the determination of the oncolytic activity of the sera of animals in the course of treatment by a particular antitumor preparation enables the effectiveness of the drug under test to be judged.

SUMMARY

An attempt was made to employ the oncolytic reaction to evaluate the efficacy of sarcolysin treatment in Guérin's rat carcinoma. The oncolytic properties of the sera were periodically investigated in rats suffering from this tumor and treated with sarcolysin. The results obtained were compared with the changes in the oncolytic properties of the serum of animals with tumors untreated with this perparation. It was demonstrated that the rise of the serum oncolytic activity in the treated animals is associated with the reduction of the tumor size. Conversely, the activity fall in the serum oncolytic activity in rats suffering from tumors not treated with sarcolysin is associated with the continuous growth of the tumor. It appears that determination of the oncological properties of the serum during sarcolysin treatment of animals suffering from tumors would enable the efficacy of this therapy on the tumor to be evaluated.

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* Original Russian pagination. See C.B. Translation.

EXPERIMENTAL BIOLOGY

THE EFFECT OF ADRENALIN ON THE DIVISION OF ASCITIC CANCER CELLS AND OF NORMAL EPITHELIAL CELLS

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In our previous research we showed that painful stimulation of an animal leads to a lowering of the mitotic activity of a subcutaneously transplanted Ehrlich's carcinoma [8] but causes no perceptible changes in the intensity of division of cells of an ascitic form of carcinoma [9].

A reactive decrease in the mitotic activity of a solid tumor has also been observed by other workers: Yu. N. Mol'kov — by excitation of mice with a sound stimulus [14], S. V. Sukhorukikh — during "motor excitation" of animals [16] and S. N. Aleksandrov — during mechanical stimulation alone by binding rats and mice to the bench [2]. It is interesting that, under the same conditions, Holmes [20] observed delay in the uptake of radioactive phosphorus by the nucleic acids of the tumor cells. S. N. Aleksandrov [2] points out, in addition, the significant fall in the number of mitoses in the cancer cells of an ascitic variety of carcinoma during painful stimulation of mice by the infliction of a burn on the skin of the thigh. He also observed inhibition of cell division in an ascitic tumor transplanted intraperitoneally during irradiation of the brain of animals. The lowering of the intensity of cell division in a malignant tumor is explained, as the author postulates [2], by the increased secretion of adrenalin as the result of excitation of the higher divisions of the sympathetic nervous system and of neurogenic stimulation of the medullary layer of the adrenals.

We know that during excitation of an animal, an increased concentration of adrenalin or of an adrenalin-like substance develops in the blood [12, 17]. The inhibitory action of this hormone on the processes of cell division has been demonstrated by a number of investigations in many normal tissues and organs, among these our own observations [4, 5, 10, 18, 19]. There is every reason to believe that adrenalin is one of the humoral links in the mechanism of regulation of cell division in the body.

It was interesting, in this connection, to find out whether adrenalin had any effect on the division of cells of pathologically abnormal tissues undergoing malignant change. This is all the more essential because many research workers now regard tumor growth as the result of the escape of normal tissue, of its metabolic processes and its cell division from the neurohumoral control, or of a considerable weakening of this control.

Investigations carried out with the aim of examining the effect of adrenalin on the growth of malignant neoplasms have given divergent results and the authors' conclusions are at times contradictory. The majority of specialists working in this field conclude, however, that adrenalin has a marked effect on tumor growth [1, 6, 11, 13], but under these circumstances mitotic activity was not investigated.

EXPERIMENTAL METHOD

We studied the effect of adrenalin on ascitic carcinoma in mice by the direct determination of changes in the division of the cancer cells. The tumor was transplanted intraperitoneally by injection of 0.4 ml of the ascitic form of Ehrlich's carcinoma. In view of the fact that adrenalin is unstable and is rapidly inactivated in the body, several injections were given to the animals. The hormone was injected subcutaneously or

TABLE 1

Mitotic Activity of the Cells of an Ascitic Carcinoma after the Subcutaneous Injection of Adrenalin, in a dose of 0.2 ml 3 times in the Course of 1 Hour

Time of taking material	Phases of mitosis				Atypical forms	Total number of mitoses
	P	M	A	T		
Animal No. 1						
Before injection of (control)	11	20	6	8	0	45
15 minutes after 3rd injection	14	29	6	9	4	62
Animal No. 2						
Before injection of adrenalin	9	21	10	12	1	53
15 minutes after 3rd injection	18	52	6	10	2	88
Animal No. 3						
Before injection of adrenalin	24	48	8	7	0	87
15 minutes after 3rd injection	26	68	14	15	2	125

Note. In this and in the subsequent tables, P, M, A and T designate pro-, meta-, ana- and telophase respectively.

intraperitoneally in a dose of 0.2 ml of a 1:20,000 solution (0.01 mg) for various periods of time. Ascitic fluid was taken from each animal before the injection of adrenalin (control) and several times afterwards. In fixed and stained films we counted 5000 cells and estimated the number of mitoses in each film. We tested altogether 42 samples of ascitic carcinoma. Furthermore, for purposes of comparison, we also determined the mitotic activity of the normal epithelium of the same animals.

EXPERIMENTAL RESULTS

In response to the subcutaneous injection of the hormone in a dose of 0.2 ml 3 times at intervals of 30 minutes, no decrease was found in the total number of mitoses in the carcinoma. On the contrary, as will be seen from Table 1, there was even a slight apparent increase in the number of dividing cells. In this experiment the material was taken 15 minutes after the third injection.

In the corneal epithelium of these mice (Table 2) a sharp suppression of mitotic activity could be observed. In some films not a single cell in a state of karyokinesis could be found in 100 fields of vision. (In the corneas of control animals an average of 150-200 mitoses was usually counted in this area of the film.)

In another group of animals adrenalin was injected 4 times in the course of 4 hours at equal intervals of time. The first sample of ascitic fluid was collected 1 hour after the first injection, the second 15 minutes after the third injection of the hormone, the third 15 minutes after the fourth injection, i.e., 4 hours 15 minutes after the first. The results obtained were the same, but were still more clearly expressed (Table 3). In this case too, a tendency was observed for the total number of mitoses to increase, especially on account of the metaphases.

On the following day the animals of the last group were again given adrenalin: 4 injections subcutaneously in the course of 2 hours (as always, ascitic fluid was taken first as a control). Material was taken 5 hours after the first injection. There was no doubt in the results obtained even at this stage.

TABLE 2

Sharp Fall in the Mitotic Activity of the Corneal Epithelium after Subcutaneous Injection of Adrenalin to Mice with Ascitic Carcinoma (corneas of control animals showed 150-200 mitoses per 100 fields of vision)

Animal No.	P	M	A	T	Total number of mitoses
1	0	0	0	0	0
	0	0	0	0	0
2	1	0	0	1	2
	0	1	1	0	2
3	0	0	0	1	1
	0	1	0	0	1

It can be seen from Table 4 that injection of the hormone under these conditions of the experiment leads, in fact, to an increase in the number of mitoses in the ascitic carcinoma, this taking place mainly as the result of an increase in the number of early stages of division, and especially of metaphases. In the corneal epithelium of these animals, on the other hand, complete suppression of cell division was observed; hardly any mitoses were found.

This experiment was performed 24 hours later on the same mice as the previous one, and it could therefore be observed that in the ascitic carcinoma films, after the increase in the number of mitoses resulting from adrenalin (Table 3), their number on the following day fell sharply (see control in Table 4).

We obtained similar results after intraperitoneal injection of adrenalin (Table 5). The hormone was injected twice into the mice of this group (the second injection was given 1½ hours after the first). Samples were taken 1½ and 3 hours from the moment of the first injection and in addition, on the following day; after this, the animal received a further injection of hormone (3 injections in the course of 1 hour at intervals of 30 minutes), and material was taken 1½ hours after the first of the 3 injections. The trend of the changes in the processes of division is illustrated in Table 5. After injection of adrenalin directly into the ascitic fluid, i.e., intraperitoneally, the number of mitoses among the

TABLE 3

The Effect of Adrenalin on Division of Ascitic Carcinoma Cells after Subcutaneous Injection of a Dose of 0.2 ml 4 Times in the Course of 4 Hours

Time of taking material	P	M	A	T	Atypical forms	Total number of mitoses
Animal No. 1						
Before injection of adrenalin	20	30	8	19	2	79
15 minutes after: 1st injection	22	41	4	15	1	83
3rd injection	23	50	4	9	2	88
4th injection	43	136	6	12	3	200
Animal No. 2						
Before injection of adrenalin	25	21	11	22	2	81
15 minutes after:						
1st injection	36	28	11	20	1	96
3rd injection	25	36	8	12	1	82
4th injection	27	70	4	8	1	110
Animal No. 3						
Before injection of adrenalin	12	9	4	4	0	29
15 minutes after: 1st injection	8	16	1	6	0	31
3rd injection	10	22	2	6	3	43
4th injection	12	32	2	4	2	52
Animal No. 4						
Before injection of adrenalin	14	15	12	24	2	67
15 minutes after:						
1st injection	—	—	—	—	—	—
3rd injection	21	48	10	22	2	103
4th injection	—	—	—	—	—	—

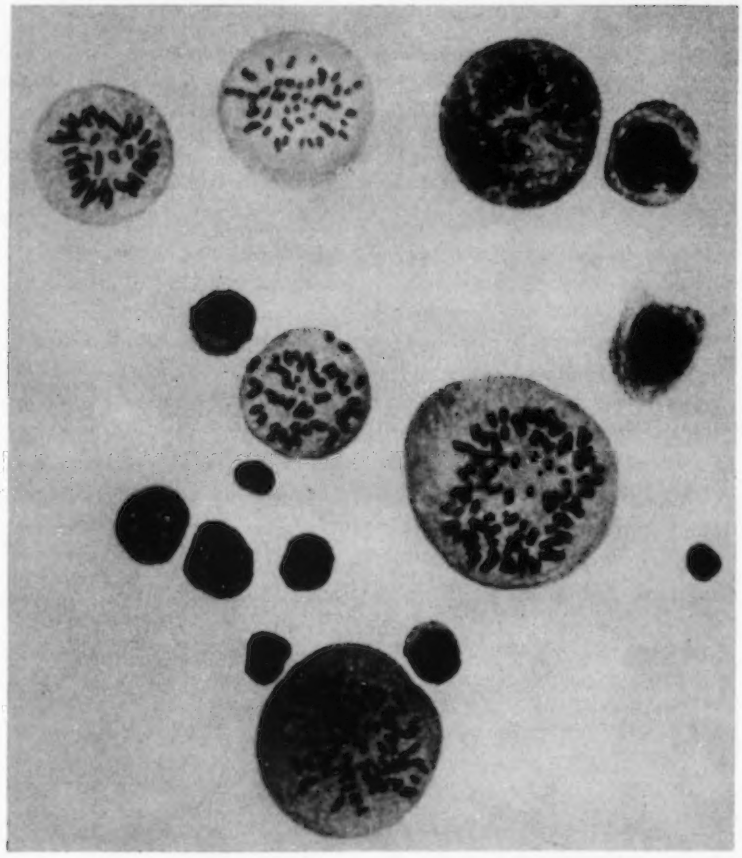
TABLE 4

Changes in the Number of Mitoses in an Ascitic Carcinoma after Subcutaneous Injection of Adrenalin in a Dose of 0.2 ml 4 Times in the Course of 2 Hours

Time of taking sample from the moment of the 1st injection	P	M	A	T	Atypical forms	Total number of mitoses
Animal No. 1						
Before injection of hormone	12	9	0	7	0	28
5 hours afterwards	26	92	14	18	5	155
Animal No. 2						
Before injection of hormone	10	2	2	2	0	16
5 hours afterwards	23	24	4	6	6	63
Animal No. 3						
Before injection of hormone	9	3	2	1	0	15
5 hours afterwards	27	32	3	7	4	73

Changes in the Mitotic Activity of Ascitic Carcinoma Cells after Intraperitoneal Injection of Adrenalin

Time of taking material	P	M	A	T	Atypical forms	Total number of mitoses
Animal No. 1						
Before injection of adrenalin	3	9	4	10	1	27
After 1st injection	5	15	3	5	1	29
After 2nd injection	12	40	6	10	3	71
On the following day	15	82	16	28	3	144
Animal No. 2						
Before injection of adrenalin	10	47	9	18	2	86
After 1st injection	18	60	18	26	1	123
After 2nd injection	22	47	12	26	5	112
On the following day	13	57	22	31	3	126
After 3 injections, each of 0.2 ml	27	110	28	25	5	195
Animal No. 3						
Before injection of adrenalin	7	14	6	10	0	37
After 1st injection	—	—	—	—	—	—
After 2nd injection	21	55	18	33	5	132
Animal No. 4						
Before injection of adrenalin	10	21	5	10	1	47
After 1st injection	10	17	8	9	2	46
After 2nd injection	23	43	10	19	2	97
On the following day	21	56	7	8	3	105
After 3 injection, each of 0.2 ml	30	127	17	41	4	219



A collection of mitoses at early stages of division, pro- and metaphases, in ascitic carcinoma cells after administration of adrenalin to a mouse.

carcinoma cells increased in all the animals, as may be seen in Table 5; this was also perceptible after the first two injections.

On the following day the number of dividing cells did not fall, as was observed when the hormone was injected subcutaneously, but continued at the raised level; this was probably due to the stronger influence of the adrenalin on the ascitic cancer cells when it was injected directly into the peritoneal cavity.

After a further injection of the hormone was given on the following day, the number of mitoses increased to an even greater degree. Mitoses were completely absent from the corneal epithelium of these animals, as in the previous experiments. Thus the adrenal medullary hormone, in the doses which we used, caused obvious changes in the mitotic proliferation of the tumor cells.

In all cases of use of adrenalin, the ascitic carcinoma films showed an increase in the number of cells in a state of karyokinesis which could be seen. A noteworthy feature was that after subcutaneous injection of this hormone, this increase always took place on account of the prophases and especially the metaphases; under these circumstances the number of late stages of karyokinesis usually showed no perceptible change. When adrenalin was injected directly into the ascitic carcinoma, a rise was observed in the numbers of all the phases of division. The changes in division in this case were more profound; this was also confirmed by the fact that the increased number of mitoses was also maintained on the following day. In our experiments we believe that adrenalin changed the processes of division in the normal tissue and the malignant tumor in the same direction but to a different degree. The tumor tissue was less sensitive to adrenalin. In the corneal epithelium of the experimental

animals the cells completely lost their ability to divide, but the mitoses which were taking place succeeded in completing themselves, whereas in the tumor the process of proliferation continued, although at a reduced speed. The course of mitoses itself was especially retarded, since the changes which took place in the cancer cells in the period of interkinesis, under the action of adrenalin, mainly affected mitosis, as the more vulnerable stage in the ontogenesis of the cell. Division, it must be thought, was even arrested, especially at the metaphase stage. This led to an increase in the number of visible mitoses in the ascitic carcinoma films.

Atypical mitoses with short, scattered chromosomes and very large, swollen dividing cells with vacuolated protoplasm were seen apparently slightly more often in the ascitic carcinoma cells after the action of adrenalin (see figure).

After giving single injections of smaller doses of adrenalin, I. A. Alov [3] observed no perceptible changes in division of carcinoma cells, whereas in the corneal epithelium he found severe suppression of division. In our investigations the tumor tissue showed lower reaction than did normal tissue to various stimuli [8, 10]. This all undoubtedly suggests that tumor cells have escaped to some degree from the neurohumoral control of the cell division of the body; they have become less sensitive to the factors and mechanisms responsible for this control, although they have not ceased altogether to react to them. It is this latter circumstance which must explain the numerous reports in the literature of the influence of the nervous system and of the endocrine organs on tumor growth.

Our investigations show that one of the main humoral regulators of cell division in the body is adrenalin, and that after its repeated administration, it quite obviously has an effect on proliferation of cancer cells. This is also suggested by the observations of other authors [1, 2].

This peculiarity of the reactivity of tumor cells which we have described cannot be reduced to a simple quantitative phenomenon, i.e., to their lower degreee of sensitivity. Disturbance of the course of mitosis itself and the accumulation of metaphases (which was never observed in the corneal epithelium) show that there is also a qualitative aspect of the reactivity of carcinoma cells which distinguishes them from normal tissue.

SUMMARY

Experiments were performed on white mice with Ehrlich's ascitic carcinoma. 1: 20,000 adrenalin solution in a dose of 0.2 ml was repeatedly injected into the animals at different time intervals.

Under the effect of adrenalin a sharp depression of mitotic activity occurred in the corneal epithelium, and mitoses were absent almost completely. Conversely, a considerable increase in the number of cells, especially at the metaphase stage was noted in the carcinoma in these animals. The latter may evidently be explained by the diminished intensity of cellular reproduction as the result of a more prolonged period required for mitosis.

Obviously the cells of ascitic cancer were less sensitive to adrenalin than those of the corneal epithelium. While the latter completely lost their ability to multiply, cancer cells continued to divide — although at a slower rate—with the course of mitosis particularly delayed.

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THE MITOTIC ACTIVITY OF THE UTERINE EPITHELIUM IN MICE AT DIFFERENT PERIODS OF PREGNANCY

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The present investigation is the continuation of previous work [2] in which it was shown that changes in the mitotic activity of the epithelium of the mucous membrane of the uterus in mice in the course of the sexual cycle may be expressed by a biphasic curve. Under these circumstances the first rise in mitotic activity is observed in proestrus and the second immediately after the end of estrus, i.e., in the first days after ovulation and the formation of the corpora lutea. The first increase in the number of mitoses during proestrus may be explained by the increased concentration of estrogens in the blood, which is in accordance with data in the literature [1]. The problem of the participation of the corpus luteum hormone — progesterone — in the regulation of the mitotic activity of the test tissue remains uncertain, for the view exists that the corpora lutea in rats and mice become functionally active only after copulation or certain other influences [5].

We decided to investigate the mitotic activity of the epithelium of the mucous membrane of the uterus in the earliest periods of pregnancy, when natural activation of the corpora lutea takes place. We were also interested in the question of parallelism in the changes in the mitotic activity of the uterine epithelium and the morphological reconstruction of the mucous membrane in connection with the formation of decidual tissue and resorption of the epithelium. We have previously shown [3] that transplantation of Ehrlich's adenocarcinoma in mice has a depressing action on mitoses in the uterine epithelium. In our research we therefore describe some results from the study of the mitotic activity in the epithelium of the uterine mucosa in pregnant mice after transplantation of a tumor.

Comparatively little work has been done on the study of the mitotic regime of the body during pregnancy. Lane [9] carried out an extensive investigation of the mitotic activity of the follicular apparatus of the ovaries in pregnant rats, and found changes in the number of mitoses in the cells of the zona granulosa; he explained these changes by variations in the quantity of gonadotropins produced by the anterior lobe of the hypophysis and the placenta. Ladman [8] showed an increase in the number of cell divisions in the anterior lobe of the hypophysis in mice, starting on the 9th-10th day of pregnancy which, in his opinion, was due to an increase in the quantity of estrogens circulating in the blood. S. S. Laguchev [4] observed a rise of the mitotic activity of the epithelium of the mammary glands in mice on the 4th, and especially on the 8th, 15th and 18th days of pregnancy. According to I. A. Alov's findings [1], the increased blood concentration of estrogens in mice during pregnancy has a depressing action on cell division in several organs not directly connected with the reproductive system (the epithelium of the cornea, tongue and intestine).

The papers cited are of undoubted interest and, on the whole, confirm the view of the important role of the estrogens in the regulation of cell division. At the same time, the problem of the participation of the corpus luteum hormone in this process during pregnancy remanins untouched in the literature.

EXPERIMENTAL METHOD

Investigations were carried out on white mice weighing 20-25 g. Altogether 200 female mice were used in the experiments; in some of these animals an Ehrlich's adenocarcinoma was transplanted subcutaneously (0.2 ml of ascitic fluid). A preliminary investigation of the sexual cycle of the female mice was made, using the method of vaginal smears, and in the stage of early estrus they were paired with male mice. A positive vaginal test was used as evidence of copulation. The inseminated females were sacrificed on the 2nd, 3rd, 5th and 8th days of pregnancy, always between 11:00 and 13:00 hours. Mice with a transplanted tumor were sacrificed on the 3rd and 8th days of pregnancy (on the 12th-14th day of growth of the tumor). Vaginal smears were taken daily throughout the experiment.

The uterus, oviduct and ovaries of the pregnant mice were fixed in Zenker's fluid. Transverse serial section (7 μ) through the upper half of the right cornu of the uterus were stained with Carazzi's hematoxylin; the number of mitoses in the epithelium of the mucous membrane was counted in every fourth section, as described in our previous paper [2]. The mitotic coefficient was determined as the number of mitoses per unit area of epithelium.

The periods of pregnancy were identified by the stage of development of the embryos, and for this purpose, in a number a cases serial sections were cut of the oviduct and uterus; the stages of development were identified from the table given in the paper by Sobotta [11-13] and Burckhard [6].

EXPERIMENTAL RESULTS

Investigation of the vaginal smears showed that even on the 3rd day after copulation all the experimental mice had passed into a state of diestrus. On the 2nd day after copulation the diameter of the uterine cornu was considerable smaller than in estrus. The lumen of the cornu was relatively large; its contours were irregular, sometimes with large inlets. The epithelial layer was mainly ill-defined from the underlying tissue. The epithelial cells were cubical and cylindrical and their nuclei usually polymorphic and irregularly stained. Signs of pyknosis and rhexis of the nuclei were frequent. Sloughing of individual cells and, less often, of portions of the epithelial layer into the cavity of the uterus were observed. The stroma was compact and its cells mainly round; the uterine glands were poorly developed. Embryos in the two-blastomere stage could be found in the oviducts at this time.

The mitotic coefficient in the epithelium of the uterine mucosa at this period of pregnancy was 0.206 (Table 1). It must be pointed out that the mitotic coefficient in normal mice during estrus, i.e., at the moment of copulation, is close to zero [2].

TABLE 1

Mitotic Coefficient in the Epithelium of the Uterine Mucosa in Mice at Different Periods of Pregnancy

Days			
2nd	3rd	5th	8th
0,206 (15)	0,282 (9)	0,005 (8)	0,000 (9)

Note to Tables 1 and 2. The number of animals is given in brackets.

On the 3rd day after copulation the surface of the epithelial layer became obviously covered with tubercles. The cells of the epithelial layer became high-prismatic; their nuclei were elongated and evenly stained. The stroma was still more compact, especially close to the lumen. The glands underwent considerable development. These changes in the endometrium were specific for the active lutein phase of the ovary and are known as progestin changes [5]. In the oviducts were found embryos at the 8-16 blastomere stage, indicating a pregnancy of 48-60 hours. The mitotic coefficient in the epithelium of the uterine mucosa was 0.282, i.e., it had altered only little in the course of the previous 24 hours.

On the 5th day after copulation the cavity of the uterine cornu was placed eccentrically, constricted and elongated in the direction of the mesometrium. The epithelial layer was quite clearly demarcated from the underlying tissue. Its cells were cubical and cylindrical, with round or slightly oval, evenly stained nuclei, displaced toward the basal part of the cell. Blastocysts lay freely in the cavity of the uterine cornu, situated in the antimesometrial or, less often, in the central portion of the lumen. From their external appearance they could be identified as belonging to the beginning or middle of the 5th day of pregnancy. The stroma near the

lumen was very compact and consisted of round cells; at the periphery they were more loosely arranged. There were large numbers of glands. The vascular network was well developed. The mitotic coefficient in the epithelium of the uterine mucosa on the 5th day of pregnancy had fallen to 0.005.

At the beginning of the 8th day after copulation embryos were found in the stage of a rudimentary allantois, surrounded by giant cells of decidual tissue. The uterine cornua were greatly thickened and the sites of implantation were clearly visible to the naked eye. The uterine cavity was displaced by the proliferating decidual tissue toward the mesometrium. This was shown in transverse sections in the form of a very small, narrow lumen, lined with a flat epithelium. Cell division in the epithelium was absent (see Table 1).

Such were the changes in the mitotic activity of the epithelium of the uterine mucosa in the early periods of pregnancy, up to its involution.

In the mice with 12-to 14-day-old tumors (Table 2), on the 3rd day of pregnancy the mitotic coefficient in the uterine epithelium was more than halved ($P = 0.05$).

TABLE 2

Mitotic Coefficient in the Epithelium of the Uterine Mucosa in Pregnant Mice with Transplanted Tumors

Description of group	Mitotic coefficient in early periods of pregnancy	
	3rd day	8th day
Healthy mice	0,282 (9)	0,000 (9)
Mice with tumors	0,125 (9)	0,000 (7)

This result agrees with our previous findings: after transplantation of a tumor into nonpregnant mice, a significant fall in the mitotic activity in the epithelium of the uterine mucosa was found on the 20th day of growth of the tumor, and on the 10th day merely a tendency for this to fall was observed [3].

It is interesting that, whereas on the 3rd day of pregnancy we observed a definite reaction on the part of the uterus to growth of the tumor (a fall in the number of cell divisions in the epithelium), cleavage of the ova in the oviducts of these mice took place at the same times as in normal animals, and no visible changes were observed in the structure of the blastomeres.

On the 8th day of pregnancy there was complete absence of mitoses in the epithelium of the uterine mucosa of the mice with 12-to14-day-old tumors, as in normal animals (see Table 2).

In normal pregnant mice we observed a slight rise in the mitotic activity of the epithelium of the uterine mucosa on the 2nd and 3rd days of pregnancy by comparison with the usual mitotic activity during estrus [2], and by the 5th day this had fallen to a very low value. Soon after this period involution of the cavity of the uterus began, with gradual resorption of the epithelium.

The findings obtained show that the changes in mitotic activity corresponded to the morphological reconstruction of the epithelium of the uterine mucosa in the early periods of pregnancy. Confirmation was thus obtained for our previous hypothesis that the mitotic regime may reflect the functional state of an organ.

On the 2nd and 3rd day of pregnancy the mitotic coefficient in the uterine epithelium was 0.206 and 0.282 respectively; these values were very close to the value of the mitotic coefficient (0.23) in nonpregnant mice during the change from estrus to metestrus, i.e., during the same periods after ovulation [2]. This suggests that in our experiments the activation of the corpora lutea after copulation, i.e., the beginning of the appearance of progesterone in the blood stream, did not affect the mitotic activity of the uterine epithelium. This does not mean, however, that progesterone in genral plays no part in the regulation of cell division.

Lloyd [10], for instance, in experiments on castrated rabbits, found an increase in the number of mitoses in the uterine epithelium by almost 50 times after administration of physiological doses of progesterone for 3 days. Progesterone, therefore, is capable of a stimulating action on mitosis in the uterine epithelium. It is also known that the "free" progesterone content of the plasma in mice increases considerably on the 7th-8th day of pregnancy [7]. It seems to us, however, that on account of implantation of the embryo and reconstruction of the endometrium, the epithelium is then in no condition to response, as a structure undergoing resorption, to the action of progesterone by an increase in the number of cell divisions. This suggest that the participation of the sex hormones in the regulation of mitotic cell division in the body is much more complex than might have thought on the basis of the existing data. In order to elucidate further the character of this influence, experiments must be performed in which estrogens and progesterone are given to castrated animals. The results of such experiments will be the subject of our next communication.

SUMMARY

In comparison with the usual mitotic activity during estrus, the mitotic activity of the epithelium uterine mucous membrane rises on the 2nd and 3rd days, decreases considerably on the 5th and ceases entirely on the 8th day of pregnancy. These changes of mitotic activity correspond to the morphological endometrial readjusment associated with the formation of decidual tissue and epithelial resorption. In mice with inoculated Ehrlich's adenocarcinoma the mitotic coefficient decreased in the epithelium of the uterine mucous membrane on the third day of pregnancy. No disturbances of the cleavage process nor changes in the blastomere structure were noted.

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MORPHOLOGY AND PATHOMORPHOLOGY

THE ULTRASTRUCTURE OF HUMAN RED CELLS

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By the use of a special method of mounting a red cell film between two films of sapon lac, Gol'din [1] showed the existence of three types of red cell stroma. In addition to red cells with a homogeneous structure, he found a stroma made up of superimposed reticular layers, and also a stroma with a large defect of the membrane, which in this author's opinion indicated the position of the nucleus.

We have observed forms similar to those described by Gol'din, by the use of a method of preparation of films suggested by Reagan and his co-workers [2] with slight modifications.

EXPERIMENTAL METHOD AND RESULTS

To two drops of blood, taken from the finger, was added 2 drops of physiological saline with heparin (10 mg heparin in 10 cm^3 of physiological saline). The mixture was kept for 1 hour in the refrigerator and for 10 minutes at room temperature and agitated from time to time. Drops of the mixture were placed on a grid and immediately aspirated into a finely drawn-out pipette.

The preparations were rinsed with 6-7 drops of distilled water and, after drying, were dusted with nichrome at an angle of 15°.

Red cells from cancer patients and healthy persons were investigated.

In these investigations we found no differences in the electron-microscopic picture of the red cells.

The total magnification (electron-optic and photographic) of the photographs illustrated here is approximately 8000 ×.

In addition to red cells with a comparatively homogeneous structure, from time to time forms were found with defects of the membrane. These defects were present to varying degrees — small, isolated holes, multiple holes — giving the stroma the appearance of net or lace and, finally, large defects in the membrane. It is thus difficult to agree with L. S. Gol'din that the large membrane defects indicate the position of the nucleus.

Among these forms of red cells others could be found which were identical with those described by L. S. Gol'din. For example, the red cell in Fig. 1, b resembles that describe by Gol'din as "Type I", and that in Fig. 1, d as "Type II". It does not seem possible to us, however, to divide them into separate types, for they are combined with homogeneous forms and also with intermediate forms (see Fig. 1).

This evidently reflects gradual changes in the osmotic resistance of the red cells in the course of maturation and senescence.

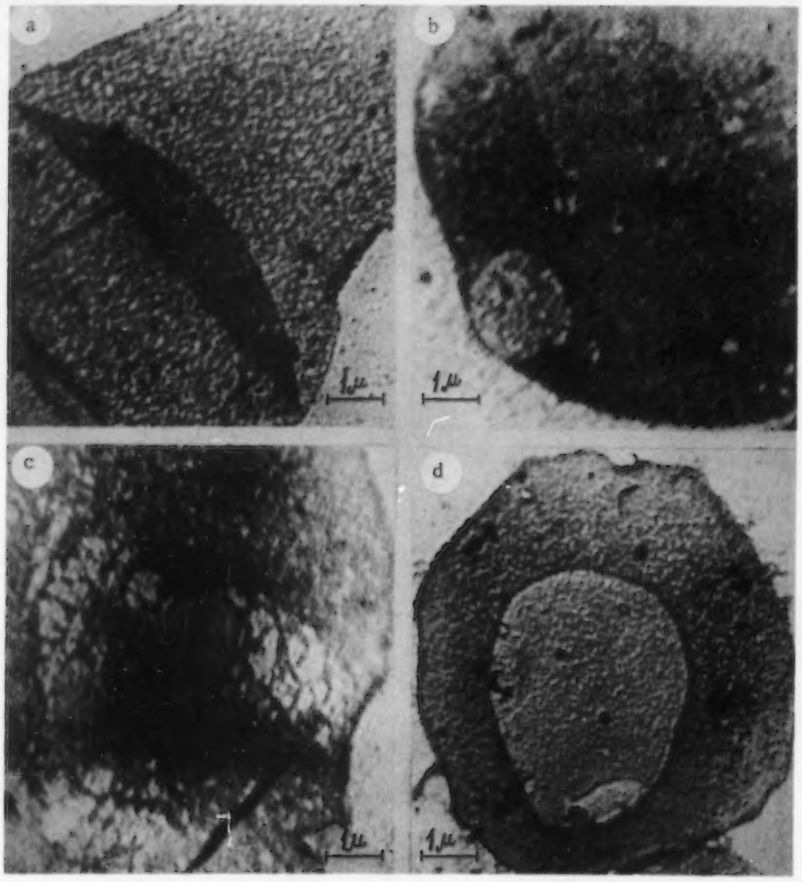

Fig. 1. Different forms of disturbance of the stroma of red cells by osmotic hemolysis. a) Diffuse rarefaction of the membrane; b) membrane in the form of lace; c) a single opening in the membrane; d) a large defect in the membrane.

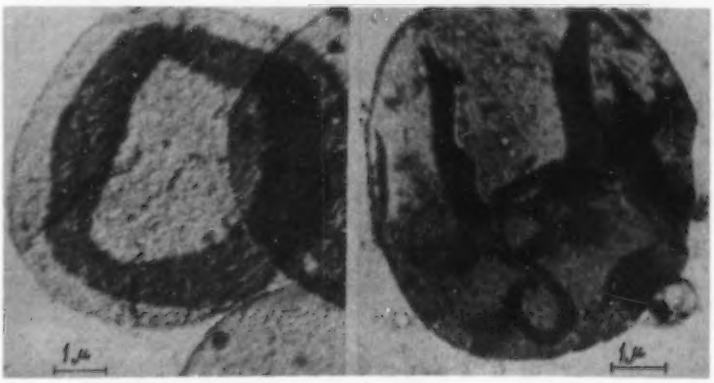

Fig. 2. Structures formed by fragments broken off the membrane and by remnants of hemoglobin.

In spite of the fact that the preparations were rinsed with distilled water, the stroma of many cells showed a greater or lesser amount of hemoglobin, in the form of clumps or granules. Together with the folds in the membrane, they formed now and then quite distinctive patterns (Fig. 2).

It is possible that the structures described by Gol'din as resembling Cabot's rings are similar in origin.

SUMMARY

Forms with defects of various degrees were discovered in an electronic-microscopic study of the erythrocytic stroma obtained by osmotic hemolysis. These forms can not be split into individual groups (as it was done by Gol'din), since they are transitionally linked with each other.

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METHODS

INVESTIGATION OF THE FRAGILITY OF THE VESSEL WALLS IN IRRADIATED DOGS BY A MODIFIED CUP TEST

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In studing the pathogenesis of the hemorrhagic syndrome in radiation sickness, investigation of the state of the vessel walls in irradiated animals are of great importance. It is essential to develop a sufficiently sensitive method of estimation of the fragility of the vascular wall (precapillary and capillary), since the accepted methods (Konchalovskii — Rumpel — Leede tournequet test, Nesterov's cup test, pinching method, etc.) are unsuitable for this purpose.

We have devised a modified cup test which enables the changes in the fragility of the vessels in irradiated dogs to be studied over a period of time.

The apparatus for measurement of the fragility of the vessels consists of an ordinary 5 ml syringe, to which is attached a short rubber tube, which in turn is connected to a small glass tube, constricted at one end (the edge of the opposite end of the tube is slightly thickened). The glass tube is connected to the rubber tube by its constricted end. So that the rubber tube does not slip off the tip of the syringe, a small blob of solder is fixed to the tip, and the rubber tube is firmly attached to the tip by strong thread or wire above the blob of solder.

The measurement of the fragility of the vessels is done by 2 persons. One everts the dog's lip and firmly applies the glass tube to the mucous membrane of the lip (Fig. 1). The other operator then moves back the plunger of the syringe from the 3 ml to the 5 ml division (in this position the plunger is fixed by the fingers) and switches on a seconds counter. After a certain time the glass tube is removed and the place to which it was applied is carefully examined in order to see whether hemorrhages develop there. The hemorrhages look like tiny red pin-points.

If hemorrhages are present the cup test is performed on a neighboring area of mucous membrane for a shorter time. The measurements are repeated until the shortest time necessary for the development of hemorrhages in the mucous membrane at the point of application of the glass tube is found.

If after the first application of the tube no hemorrhages appear, the time for application of the glass tube to the mucous membrane is gradually lengthened until hemorrhages are found in the mucous membrane.

For reasons of economy of time, it is more convenient to do the cup test at the following "standard" time intervals: 1, 2, 3, 4, 5, 10, 15, 30, 45, 60, 90, and 120 seconds. If the cup test result is still negative after 120 seconds, no further measurements should be made and the value given as "over 120 sec".

The shortest interval of time in the course of which hemorrhages appear at the point of application of the glass tube to the mucous membrane (with a standard degree of rarefaction of air in the tube system) gives the value of the cup test.

In healthy dogs the value of the cup test is usually from 5 to 90 seconds.

In the experimental animals the investigations of the cup test were carried out during a period of 3-10 days before irradiation, and then every day after irradiation. The dogs were subjected to total γ-irradiation from a cobalt source (Co^{60}).

Fig. 1. Investigation of the fragility of the capillaries in a dog by the modified cup method.

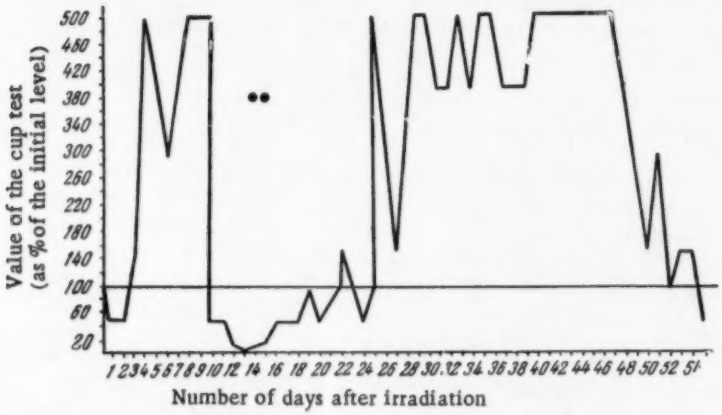

Fig. 2. Changes in the cup test in a dog irradiated with a dose of 200 r. The valvesof the cup test which exceed 120 seconds are conventionally taken as 500%. The dots indicate the presence of hemorrhages in the skin or mucous membranes on the corresponding days.

with air doses of 100, 200, 300, 400, 500, 600, 700, 1000 and 5226 r. The conditions of irradiation in all cases were the same, apart from the exposure.

Depending on the dose, the exposure varied from 13 minutes 33 seconds (100 r) to 12 hours 9 minutes 12 seconds (5226 r).

The results of the investigation of the cup test in irradiated animals are shown in Figs. 2 and 3.

The distinctive reaction of the vessel wall of the irradiated animals should be noted. The resistance of the vessel wall in the first few days after irradiation increases irrespective of the dose of irradiation (within the limits of the test doses). The increase in the resistance usually reaches its maximum on the fourth day after irradiation The appearance of hemorrhages is preceded by a fall in the value of the cup test far below its initial level.

It is interesting to note the presence of the following tendency: the higher the dose of irradiation, the shorter the period of increased resistance of the vascular wall.

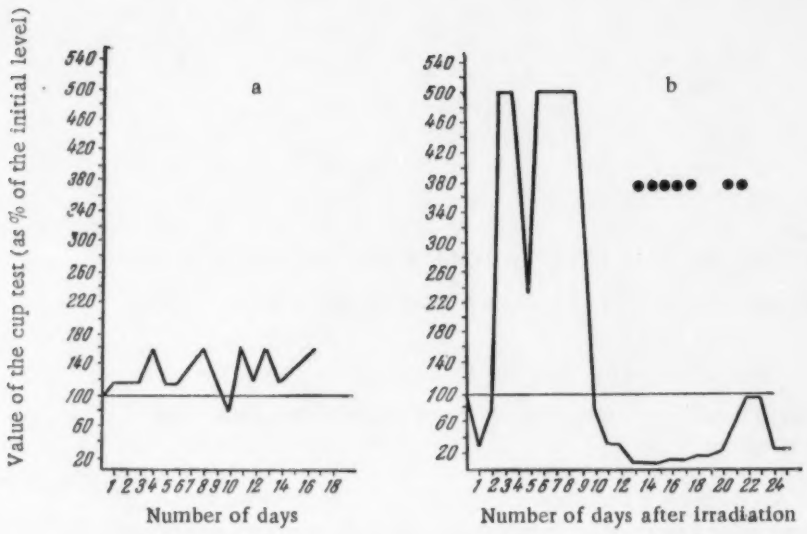

Fig. 3. Changes in the cup test in a dog irradiated with a dose of 300 r. a) Control; b) irradiated dog. Remaining legend as in Fig. 2.

SUMMARY

A modified cup test is suggested for investigating the fragility of the vascular wall in dogs. The method permits a follow-up under dynamic conditions of the actual strength of the vascular wall in irradiated dogs, which is impossible with any other method of examination (the tourniquet method of Konchalovskii-Rumpel-Leede, Nesterov's cup test etc).

A technique of free skin grafting in small laboratory animals is depicted along irradiation, irrespective of the irradiation dose with its notable decrease in the period preceding hemorrhages.

REGISTRATION OF THE RESPIRATORY MOVEMENTS IN MAN DURING PHYSICAL WORK BY MEANS OF A THERMOBATTERY

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(Received February 17, 1958. Presented by Active Member AMN SSSR A. I. Nesterov)

The registration of respiration in man during physical exertion presents certain difficulties, for when pneumatic, powdered carbon or piezoelectric transmitters are used, mechanical distortion of the curve takes place.

There are reports in the literature of the registration of respiration in rabbits by means of a thermobattery, making use of the difference in the temperature of the inspired and expired air. In the registration of respiration by this method the thermobattery is fixed to the nose of the animal by means of a small rubber mask. This method introduces no external resistance to the act of respiration.

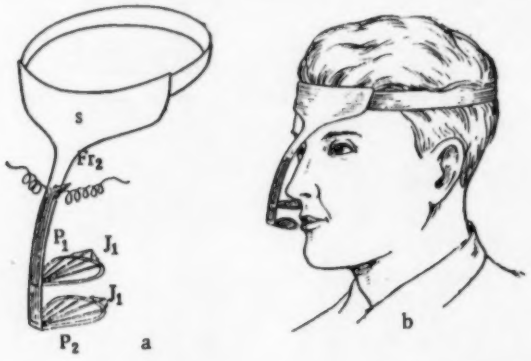

Fig. 1. Scheme (a) of the apparatus for registration of respiration in man by means of a thermobattery and (b) the method of attaching it.

In order to make use of this principle in the registration of the respiratory movements in man during physical effort, we introduced a few modifications into the construction and method of attachment of the battery as used in rabbits.

The method is as follows (Fig. 1). To the subject's forehead a T-shaped support S, made of half-millimeter aluminum sheet, is fixed by means of an elastic band. To the lower part of the support, plastic arms P_1 and P_2 are fixed against the mouth and nostril. On the plastic arms are arranged copper – constantan thermocouples, connected in series to form a battery, so that some junctions (working) J_1 are opposite the nostril and mouth, and the other junctions J_2, corresponding to them, are collected together at a point a few centimeters above, where they are not exposed to the action of the expired air current.

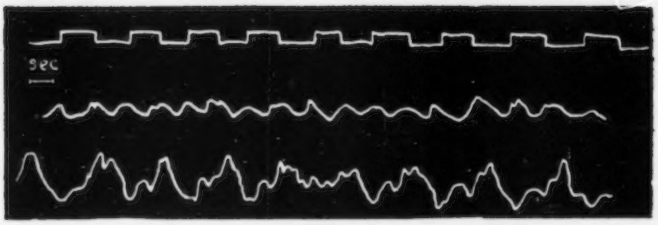

Fig. 2. Simultaneous registration of respiration by means of a thermobattery and a piezoelectric generator during physical exertion. Significance of the curves (from above down): rhythm of physical exertion, recording of respiration by means of the thermobattery, tracing of respiration by means of a piezoelectric generator.

Because of the differences in the temperature of the expired and environmental air, an electromotive force is generated in the thermobattery, which is transmitted via an amplifier to a recording system. For this purpose we used the 4-PFD-7 functional diagnostic apparatus of the experimental factory VNIIMI and O of the Ministry of Health of the USSR.

For comparison, in Fig. 2 are shown the recordings of respiration obtained by means of a thermobattery and piezoelectric generator in a human subject during physical exertion (raising and lowering a weight of 6 kg by flexing the lumbar spine).

As may be seen in Fig. 2, the working movements do not affect the true recording of respiration by means of the thermobattery; in the recording obtained by means of the piezoelectric generator, which is fixed to the abdomen, it is mainly the mechanical oscillations of the work rhythm that are shown.

SUMMARY

One of the junctions of the thermobattery is fixed against the nostrils and mouth of the subject under investigation. The process of respiration gives rise to an EMF in the thermobattery, which though an amplifier is transmitted to the registering instrument. Movements performed by the subject under investigation during work have no distorting effect on the respiration recording.

GRADUAL EXPERIMENTAL CLOSURE OF THE LUMEN OF THE PORTAL VEIN

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(Received December 15, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

In experimental investigations on the liver it is sometimes necessary to ligate the portal vein.

The one-stage ligation of the portal vein is known lo lead to the rapid death of the animals: dogs, cats, rabbits and rats die from 30 minutes to 15 hours after ligation [1, 5, 6, 8, 9, 12]. In carrying out chronic investigations it is essential that an animal in which the portal vein has been ligated should live for a long time. The methods of ligation of the portal vein that have so far been suggested and which permit survival of the animal may be divided into two groups.

The first group includes operations in which the branches of the portal vein — superior mesenteric, gastro-epiploic and pancreaticoduodenal veins — are ligated in stages [3, 6, 11]. Multiple operations under ether anesthesia are not without effect on the animal, especially when the liver is excluded. The operation wounds heal badly and the repeated operations leave adhesions which render manipulations of the portal vein difficult.

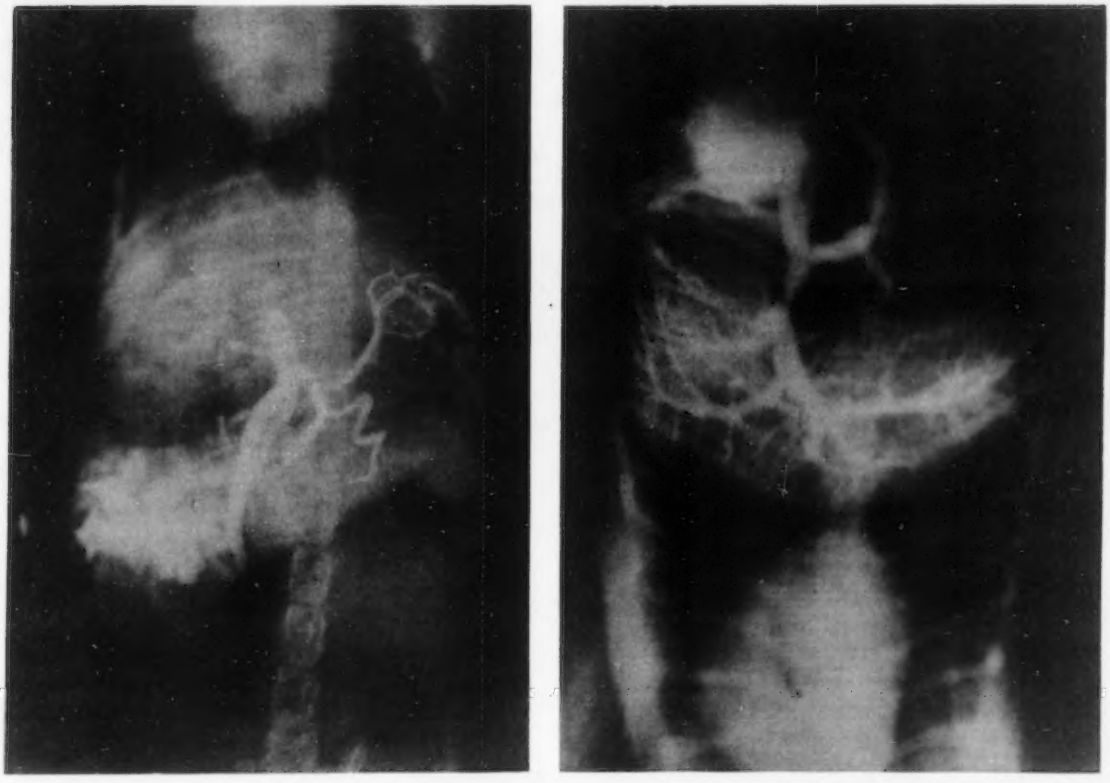
The second group consists of operations to close the lumen of the portal vein gradually [1, 2, 3, 8]. Many authors solve this problem differently. The original method was suggested by N. N. Burdenko [1]. He ligated the pancreaticoduodenal vein and constricted the gastrosplenic vein. Around the trunk of the portal vein he passed a loop of thread, which was fixed behind the deep spinal muscles. It was assumed that when the animal got up, the contracting muscles would draw the thread tight and constrict the lumen of the vein. From 1 to $1\frac{1}{2}$ months later, and sometimes a little before, the portal vein was completely ligated. This author did not carry out a check of complete obstruction of the portal vein.

Recently experimental workers have mainly used the method of constriction of the lumen of the portal vein in stages. Some authors [4, 6, 8] assert that the first constriction of the portal vein should only be to an insignificant degree, but others [2, 7] point out that in some cases the first constrictions may be by more than half the lumen of the vein.

The majority of workers use a silk ligature. A silk ligature, however, even after prolonged stenosis of the portal vein, as a rule fails to close the lumen completely, and repeated ligation has to be done. There are no convincing reports in the literature giving the times of complete closure of the lumen of the portal vein by the different operative methods.

In our own experimental work on the study of the effectiveness of portocaval anastomosis between the left kidney and the omentum, it was necessary to produce artificial portal hypertension in dogs.

For this purpose, in 6 dogs we first used N. N. Burdenko's method [1], but we found no indication that this method had been used by anybody else besides N. N. Burdenko himself. In all 6 dogs, 5-6 months afterwards, splenoportography showed that the lumen of the portal vein was not completely closed, and the development of preexisting and of newly formed portocaval anastomoses was quite inadequate.



Closure of the lumen of the portal vein in an experiment on a dog.

There are indications in the literature of the use of an aneroid cuff and of a cotton tape, sprinkled with decithyl phosphate powder for gradual closure of the lumen of the vessel [10]. Since these materials were not available, we used cotton thread soaked in iodine solution.

After only 10-13 days the lumen of the portal vein was completely closed, as was demonstrated by splenoportography and at postmortem. The experimental method was as follows. From 18-20 white cotton sewing threads No. 30-40 a string was made, 70-80 cm long, the threads not being plaited. Every 20 cm, along the string, were tied two knots, separated by a short interval. The string was loosely wound on a glass tube or glass slide not more than twice, boiled in water for 30-40 minutes and then immersed in a 10% solution of iodine, in which it was kept for 8-10 days.

At operation, a ligature was cut from the string between two knots, and this was passed under the portal vein with a Deschamps' needle in the region of its bifurcation, nearer to the liver and passed to an fro under it several times. Under these circumstances the vein was twisted by the string and the adventitia was partially removed by it. The ligature was tied over a Roser – Sinitsyn bougie with the end sawn off. The lumen of the portal vein, which in dogs weighing 20-39 kg is usually 1.5-1.7 cm, was constricted in this way to 0.2-025 cm.

It must be pointed out that, in order to avoid adhesions, careful protection of the operation field and the surrounding organs with gauze from the ligature, soaked in iodine, is essential. After each operation a control splenoportography was performed to test the degree of constriction of the lumen of the portal vein.

The primary constriction of the portal vein which we performed was much greater in degree than that described by other authors.

In order to ascertain the time required for complete closure of the lumen of the portal vein by our method, we carried out splenoportography at different times in 76 dogs. In all the dogs, 13 days after application of the cotton ligature soaked in iodine, the portal vein was no longer patent. In order to determine more accurately the times of closure of the portal vein, we also sacrificed 10 dogs at various times. Hence it was found that in 2 dogs sacrificed 10 days after operation, the patency of the portal vein was still partially preserved, and in 2 dogs sacrificed on the 11th day, the lumen of the portal vein was no more than 1 mm. Of 2 dogs sacrificed on the 12th day, in one the portal vein was completely closed, and in the other there was a lumen of less than 1 mm. In 4 dogs sacrificed on the 13th day, the lumen of the portal vein was completely closed.

Our method of gradual closure of the lumen of the portal vein this enabled repeated operations to be avoided and guaranteed closure of the lumen of the vessel within a certain time.

SUMMARY

A method of gradual closure of the portal vein lumen is suggested for creating experimental portal hypertension. Portal vein is constricted to the diameter of 0.2-0.25 cm by means of a bundle of cotton threads (No. 30-40) impregnated with 10% iodine solution. When a bundle of threads is passed under the vein it is pulled back and forth several times, so as to traumatize the adventitia. With the aid of splenoportography it was established that in 76 live dogs and at autopsy in 10 dogs closure of the portal vein lumen occurred on the 13th day after iodized thread ligation of the vein.

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* As in original - Publisher's note.

SOME PECULIARITIES OF SKIN HOMOTRANSPLANTATION IN SMALL LABORATORY ANIMALS

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(Received December 29, 1958. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

A convenient object for the study of problems of tissue incompatibility arising during transplantation of organs and tissues is homotransplantation of the skin in laboratory animals.

Methods of free grafting of the skin in man are widely discussed in the literature [1-3, 5, 10]. Transplantation of the skin in laboratory animals has certain differences from free skin grafting in man, which are due to the special features of the structure of the skin in animals. Nevertheless, problems of transplantation of the skin in laboratory animals have been examined very inadequately [9, 11]; in particular there is no description of a single operative technique, on which depends, to a large extent, the fact that the results obtained by different workers during homotransplantation are dissimilar. We shall describe the operation of transplantation of the skin in rats, believing that this will help to secure standardization of the method of free grafting of the skin in laboratory animals.

Auto- and homoplastic transplantation of the skin were performed by the same method, so that one description of the method will suffice. On the day before operation the hair was clipped in the region of the operative field from an area much larger than the flap to be transplanted, so that the fur would not fall into the wound during the operation. We did not shave the skin, since the scabs which form at the site of injury to the epithelium make it difficult to assess the state of the transplantates later on. On the day of operation the operative field was twice treated with 70° alcohol. The operation was performed under sterile conditions and under ether anesthesia. Full thickness skin flaps were used for transplantation. The subcutaneous and muscle layer acted as base of the wound.

Lightly with a scalpel the border of the chosen area of the donor's skin was marked out, and the same done with the recipient. Next, the edges of the cut skin were seized with forceps of hemostats and, lifting the flap steadily, the skin was separated with a scalpel or scissors from the underlying subcutaneous and muscular layer. The skin is more easily separarated in the direction from head to tail (Fig. 1). If care is used in separating the skin and no injury is caused to the subcutaneous and muscular layer, the whole operation may be carried out almost bloodlessly. Only pinpoint hemorrhages was observed from the divided vertical branches joining the superficial and deep vascular plexuses. If the subcutaneous and muscular layer were injured during operation, careful hemostasis was secured to prevent hematoma formation and detachment of the graft. After separation of the graft and preparation of the bed, the skin flap was transferred to the recipient and placed so that the direction of growth of the hair on the graft was opposite to or perpendicular to the direction of growth of the surrounding hair. Thanks to this, the transplantate was readily identified from the surrounding tissue. The graft was fixed to the skin with a continuous suture.

Particular attention must be drawn to the fact that no underlying tissue from the subcutaneous and muscular layer must be left on the graft, otherwise the survival of the transplanted tissue is jeopardized. This can be seen

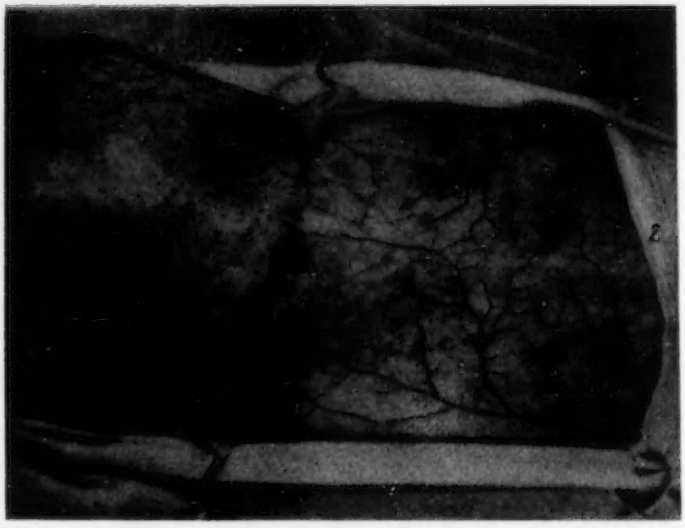

Fig. 1. Separation of the flap during free grafting of the skin in a rabbit. 1) Separated skin flap; 2) underlying subcutaneous and muscular layer.

Results of Skin Transplantation

Survival of transplantates	Transplantates			
	with subcutaneous muscular layer		without subcutaneous muscular layer	
	in rats	in rabbits	in rats	in rabbits
Survived	0	2 (partially)	30	20
Did not survive	11	8	0	0

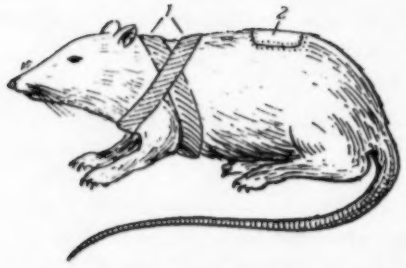

Fig. 2. Scheme of the application of the plaster bandage. 1) Plaster bandage; 2) transplantate.

from the table, in which are shown the results of auto-transplantation of the skin, depending on whether the subcutaneous muscular tissue was left on the graft or was removed from it.

To obtain better fitting of the graft to the underlying tissue, the skin flap was cut slightly smaller than the wound surface of the recipient, for this ensured slight stretching of the graft. In addition a pressure dressing was often placed over the transplantate, and fixed to the surrounding skin with silk ligatures. The sutures were taken out on the 10th-12th day after operation. In order to prevent the animals (especially the recipient rats) from biting the grafts, a light crossed plaster bandage was applied to the recipients in the region of the neck and chest, which restricted the movements of the head and body of the animal (Fig. 2).

The viability of the auto- and homotransplantated was assessed by their clinical condition (consideration was paid to the color, consistency, mobility, character of growth of the hair of the transplanted skin flaps), and also by the state of vascularization of the transplantated by the capillaroscopy method [4, 12, 13] and by making incisions in the graft..

For capillaroscopy the animal was fixed to the bench. An area of the transplantate, free from pigmented areas (or dark colored hair), which greatly interfere with the capillaroscopic examination, was shaved. The hair growing on the graft was trimmed. Next, in order to make the skin more transparent, a few drops of liquid mineral oil were placed on the graft, cedarwood, clove or bone oil may also be used [6, 10]. From 10 to 15 minutes afterwards the examination was carried out by means of a binocular loupe MBS-2 or an ordinary capillaroscope, mounted on a universal physiological stand. The capillaroscopic examination was made at intervals of 1-2 days after the 2nd-4th days after the transplantation. Disturbance of the circulation in the transplanted skin flap was regarded as an indication of death of the transplantate.

In order to assess the state of the circulation in the transplanted skin flap by the incision method, superficial epidermal scarification, 0.3-0.5 cm in length, was carried out with a razor on the graft. When the circulation in the graft was functioning, the incision was quickly filled with blood flowing from the divided vessels.

SUMMARY

A technique of free skin grafting in small laboratory animals is depicted along with various methods of evaluating the condition of the transplantates.

Skin transplantates devoid of subcutaneous muscular layer are recommended.

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ERRATA

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

Volume 47, Number 1, January 1959

Page	Line	Reads	Should Read
68	Table 2, 1st column of numbers, 6th number	+ 6, 9	+ 6, 9
107	15 from bottom	adrenalin	novocain
Volume 47, Number 4, April 1959			
402	Table 2	left retina light dark light dark	left retina dark light dark light
Volume 47, Number 5, May 1959			
Table of Contents	Title for 3rd article	The Problem. . . Shock	The Problem of the Nature of Spinal Shock. Communication III. Effect of Potassium and Calcium Ions on the Development of Spinal Shock
531	1st line of title	omitted	The Nature of Spinal Shock

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LÉM	Laboratory for Experimental Morphogenesis
LÉNDVI	Leningrad Inst. of Dermatology and Venereology
LÉO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIÉM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

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